INVESTIGATION OF AN INVASIVE ANT SPECIES: *Nylanderia fulva* COLONY EXTRACTION, MANAGEMENT, DIET PREFERENCE, FECUNDITY, AND MECHANICAL VECTOR POTENTIAL

A Dissertation

by

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ABSTRACT

Invasive species often threaten biodiversity and environmental processes in their introduced range by extirpating native species due to competition for resources. *Nylanderia fulva* (formerly *N*. (=*Paratrechina*) sp. nr. *pubens*) is an ecologically dominant and economically important invasive species in the United States. This dissertation addresses aspects of the biology, behavior, management, and collection techniques for *N*. *fulva*. Specifically, topics investigated include a modified drip technique for extracting ants from their substrate, the effectiveness of a broadcast ant bait as a stand-alone treatment, the foraging preference and peak activity of workers, the reproductive potential of queens, and the ability of this species to translocate pathogenic microorganisms. The primary goal of these works was to better understand the biological idiosyncrasies of this species that may ultimately lead to the mitigation *N*. *fulva* populations.

A modified drip technique was developed to quickly and efficiently extract *N*. *fulva* from their nesting substrates. Ants and their associated substrates were collected in 18.9 L buckets lined with talcum powder and transported to the laboratory. Substrates were weighted down and a cardboard tower was provided for the immigration of ants as they were forced out of substrates with a slow influx of water.

Three applications of Advance® Carpenter Ant Bait (ACAB) were applied to a *N*. *fulva* population in East Columbia, TX. A series of GIS interpolated maps depict achieved management and subsequent rebound of *N*. *fulva* populations. As great as 77%

population reduction was achieved by 1 week post treatment, but *N. fulva* populations rebounded within 3-4 weeks. As a stand-alone treatment, this bait did not provide adequate ant management in treatment plots.

Diet preference experiments were performed using artificial diets and food lures. These results of these trials indicated that *N. fulva* preferred the most carbohydrate rich diet offered through all seasons and that mint apple jelly or hot dog slices were the favored food lures. Diel foraging behavior was observed when temperatures were between 9.95 and 37.26°C. Peak foraging activity occurred at 28.24 ± 3.12°C.

A laboratory investigation of *N. fulva* suggested that as the number of queens increased, individual queen fecundity increased. This phenomenon is a novel observation among ants and suggests an alternative mechanism for intracolony dominance. Hexagyne colony fecundity of 0.25 ± 0.12 eggs/queen/hr was the maximum fecundity observed.

Results of laboratory experiments showed that *N. fulva* were capable of transferring *E. coli* up to 4.5 m in 6 hrs after acquisition from a contaminated source. Pyrosequencing of ectomicrobial assemblages revealed a suite of 518 bacteria and 135 fungi species associated with *N. fulva*, many of which are known pathogens of plants and animals, including humans. These results suggested that *N. fulva* should be regarded as both a medically and agriculturally important species.

DEDICATION

I would like to dedicate these works to my family, whose unwavering support and love have always been a source of my strength. Thank you for instilling within me a sense of work ethic, morality, and faith in God. Mother, your everlasting love and encouragement I carry with me always. I owe my skepticism of the implausible and sense of curiosity to your example. Dad, I hope that I display even a fraction of the selflessness that you show towards your children. Your altruistic behavior is a marvel among the ants. Glen, your unquenchable thirst for knowledge is a wonderful example, and I am happy to carry on that torch. Ray, your passion for your family and admirable career choice as a protector of the innocent are truly inspirational. Thank you for being my guardian, supporter, confidant, and comic relief. Grandma and Granddad, your constant approval and encouragement have always meant the world to me. My love, Michelle, I look forward to a lifetime of adventures with you.

Proverbs 6:6-8
Go to the ant, O sluggard;
Observe her ways and be wise,
It has no commander,
No overseer or ruler,
Yet it stores its provisions in summer,
And gathers its food at harvest.

"The Creation, whether you believe it was placed on this planet by a single act of God or accept the scientific evidence that it evolved autonomously during billions of years, is the greatest heritage, other than the reasoning mind itself, ever provided to humanity."
"Science and religion are the two most powerful forces of society. Together they can save the Creation."
~ Edward O. Wilson from The Creation: An Appeal to Save Life on Earth.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Invasive Ant Species

Many of the ecological niches that ants occupy, and the behavior associated with them, have positive effects on ecosystems. Ants serve as soil aerators, undertakers, predators, and they play a major role in the global food web (Hölldobler and Wilson 1990). However, ants are also considered to be nuisances when interfering with human activities. Ecologically, some invasive ant species are known to reduce native ant diversity, displace other arthropods, negatively affect vertebrate populations, and cause crop loss as a result of symbiotic relationships with hemipteran insects (Suarez et al. 2010). Thus, introduced species have caused extensive economic damages and ecological changes (Simberloff 2003a).

There are an estimated 15,000 – 18,000 ant species worldwide. Currently ~12,567 have been described (Gusmao et al. 2011), and invasive ant species currently comprise < 1% of the known species of ants (Suarez et al. 2010). Five ant species have been placed on the list of the 100 worst alien plant and animal species including *Linepithema humile* (Argentine ant), *Pheidole megacephala* (big headed ant), *Anaplolepis gracilipes* (yellow crazy ant), *Solenopsis invicta* (red imported fire ant, RIFA), and *Wasmannia auropunctata* (little fire ant) (Lowe et al. 2000).

Second in importance to habitat loss, invasive species threaten the biodiversity and environmental processes in their introduced range by directly and indirectly causing

extirpation of native species (Holway et al. 1998, Lowe et al. 2000, Zimmer 2001). The suppression of future introduction of ant species and management of currently known non-native invaders is essential to the conservation of the biodiversity of Earth. A comprehensive understanding of the life cycles, social structures, and ecological roles of invasive ants is fundamental to achieve proactive management strategies for future invasions.

Unfortunately, the majority of information regarding the biology and ecology of invasive ants is derived from our understanding of only two intensively studied species, *S. invicta* and *L. humile* (Suarez et al. 2010). This limits the inferences that can be made about invasive ant species as a cohesive group. There is a suite of characteristics that are commonly found in invasive ant species: they tend to be highly polygynous, unicolonial, utilize ephemeral nesting sites, quickly abscond when disturbed, reproduce by budding, are among the smallest sized members of their genus, and are behaviorally dominant over other ant species (Hölldobler and Wilson 1990, Passera 1994, Suarez et al. 2010). Determining how to exploit these characteristics may be the key to minimizing the effects of invasive ant species.

Additionally, invasive ant species often increase their interspecific competition in their introduced range (Holway and Suarez 1999). Through interference and exploitative competition, worker ants are able to discover and recruit nest mates to food more quickly and in higher numbers than native species (Holway and Suarez 1999). Typically, the lack of natural enemies in an invasive species' introduced range is also believed to improve their success (Holway et al. 1998, Holway and Suarez 1999,

Zimmer 2001). Furthermore, multiple introductions are commonly implicated in the success of biological invaders as a result of multiple sources of genetic variation (Suarez and Tsutsui 2008).

Not all introduced species are successful invaders, and often go unnoticed unless they reach pest status. There have been several explanations as to why some introductions succeed and others fail. The "biotic resistance hypothesis" states that the success of an invasive species is dependent on the ability for resident biota to resist the invasion (Simberloff 1986).

Most invasive species come from South America (Suarez et al. 2010). It is a possibility that the environmental conditions in South America favor the evolution of traits favorable for successful invaders (Suarez et al. 2010). However, it may also be biased because of the extensive biodiversity studies describing South American species (Suarez et al. 2010). It should be noted that *L. humile* and *S. invicta*, arguably the two worst North American invasive ant species, are from South America.

The loss of intraspecific aggression due to a genetic bottleneck has been implicated in the success of some invasive ant species. This has been particularly well described for *L. humile* (Holway et al. 1998, Tsutsui et al. 2000). The lack of intraspecific aggression is characterized by the abandonment of territorial behavior, fusion of spatially separate colonies, decreased worker mortality, greater foraging activity, an increase in brood production, and elevated population densities (Hölldobler and Wilson 1990, Holway et al 1998). The absence of intraspecific aggression in *L. humile* has been shown to occur with colonies spatially separated by distances up to 80 km (Holway et al. 1998). Tsutsui

et al. (2003) discovered that the effects of the genetic bottleneck are increased when individuals from less genetically diverse colonies attack individuals from more diverse colonies. This is presumably a result of the increased population density of less genetically diverse populations. Attackers survived aggressive encounters more than six times as often as the more genetically diverse recipients of aggression (Tsutsui et al. 2003). Furthermore, Argentine ants regularly perform up to 90% queen execution just prior to the production of new reproductives (Tsutsui and Suarez 2003). This releases the surviving queens from pheromonal inhibition of egg laying, and promotes social cohesion within Argentine ant colonies (Tsutsui and Suarez 2003).

Taxonomy and Origins of *Nylanderia* sp.

In 2010, LaPolla et al. revised the *Prenolepis* genus group and moved all *Paratrechina* species to the genus *Nylanderia* or *Paraparatrechina*, with the exception of *Paratrechina longicornis*. *Nylanderia* is currently in the process of revision (LaPolla et al. 2011). There are currently 130 species of *Nylandria* recognized and they are commonly the most abundant ant species in communities where they occur (LaPolla et al. 2011). Historically, the most economically important species of the genus *Nylanderia* in the Americas are *N. bourbonica*, *N. fulva*, and *N. vividula* (Fox et al. 2007, Trager 1984). No common names of species within the *Nylanderia* genus are currently recognized by the Entomological Society of America.

Auguste Forel originally described *Nylanderia pubens* in 1893 from the Caribbean island of St. Vincent (Wetterer and Keularts 2008). In 1905, *N. pubens* was recorded in

Bermuda, and a second Forel record is reported form the island of Martinique in 1912 (Wetterer and Keularts 2008). Marlatt (1928) suggested that *N. pubens* is actually a native of Brazil and not the Caribbean islands. In 1951, D. R. Smith reported that the range of *N. pubens* included Mexico, Central and South America, and the West Indies (Wetterer and Keularts 2008). Current records indicate that *N. pubens* can also be found on the islands of Anguilla, Guadeloupe, St. Croix, Puerto Rico, and Cuba (Wetterer and Keularts 2008). In the United States, *N. pubens* was first recorded in Miami in 1953, but the earliest record of this species reaching pest status was not reported until 1990 (Deyrup et al. 2000, Klotz et al. 1995, Trager 1984, Wetter and Keularts 2008). *N. pubens* is commonly referred to as the hairy crazy ant in some publications (Deyrup et al. 2000, Wetterer and Keularts 2008) and the Caribbean crazy ant in others (Warner and Scheffrahn. 2003).

Nylanderia fulva is a Brazilian species, referred to in the literature as "crazy ants". They were introduced into Boyacá Columbia between 1969-1970 as a biological control agent against *Atta* sp. and snakes in coffee growing and cattle breeding regions (Zenner de Polania 1990). As a result of the mutualistic association between *N. fulva* and *Antonina* sp. (Hemiptera: Pseudococcidae) the biological control agent became a tremendous economic pest, subsequently contributing to the desiccation of rangeland grass, and also blinded calves (Zenner de Polania 1990). After ~20 years, *N. fulva* reached population density equilibrium within the Boyacá ecosystem and was no longer problematic. However, throughout the 1970's and 1980's multiple introductions of *N. fulva* were made throughout Columbia where they were reported to prey on chickens,

other birds, and domestic animals (Zenner de Polania 1990). Subsequent death of these small animals was attributed to asphyxiation. Larger animals were also attacked around the eyes, nose, and hoofs. Furthermore, incredible population densities made this species a formidable household pest (Zenner de Polania 1990).

In 2002, Tom Rasberry, a pest management professional, discovered a population of an unknown pest ant near Pasadena (Harris County), Texas. In 2005, a second population was found in large densities that dominated other ant species throughout a residential landscape in Deer Park, Texas (~6.7 miles from the original infestation). Jason Meyers began conducting his doctoral research regarding the identification of the ant and their management. Using morphometric and phylogenetic techniques, Meyers (2008) determined that the ants were a new exotic invasive pest ant species to Texas. His results led to a tentative designation of the Texas pest ant species as *Nylanderia* (formerly *Paratrechina*) sp. nr. *pubens* (Rasberry crazy ant, RCA).

One notable difference between *N*. sp. nr. *pubens* and *N. pubens* specimens from Florida was the appearance of only two pairs of machrosetae on the mesonotum of *N*. sp. nr. *pubens*, whereas *N. pubens* has 3-4 pairs (Meyers 2008). Based on molecular comparisons, Zhao et al. (2012) proclaimed that *N*. sp. nr. *pubens* from Texas and Florida were the same species and should be regarded as *N. pubens*. Gotzek et al. (2012) identified *N*. sp. nr. *pubens* from Texas, Louisiana, Mississippi, and northern parts of Florida as *N. fulva*. They also hypothesized that most, or even all, *N. pubens* in Florida are *N. fulva*, and suggest that *N. pubens* does not possess the invasive capabilities of *N. fulva*. Furthermore, Gotzek et al. (2012) determined that *N. pubens* and *N. fulva* workers

are morphologically indistinguishable, and that species identification is only possible through examination of the male parameres or by genetic comparisons. This dissertation will consider the specimens from Texas as *N. fulva* as suggested by Gotzek et al. (2012). The revision of *Nylanderia* by LaPolla is a much needed contribution to the study of these species. Vouchered specimens from studies regarding *Nylanderia* should all be reidentified using LaPolla's forthcoming keys in order to verify past results. For example, Wheeler and Wheeler (1985) identified *N. fulva* from "extreme southern Texas" (O'Keefe et al. 2000). Given the absence of a population explosion of *N. fulva* in the 1980's, their identification is questionable and voucher specimens should be reexamined.

Biology and Behavior of *N. fulva*

Nylanderia fulva is a successful invasive species in Texas. This is presumably a result of its omnivorous feeding habits, polydomous nesting, extreme polygyny, high fecundity, high interspecific aggression, and apparent lack of intraspecific aggression. Extraordinary population densities of *N. fulva* have been found in localized infestations within 24 Texas counties that include Bexar, Brazoria, Brazos, Cameron, Chambers, Comal, Fort Bend, Galveston, Hardin, Harris, Hidalgo, Jefferson, Jim Hogg, Liberty, Matagorda, Montgomery, Nueces, Orange, Polk, Travis, Victoria, Walker, Wharton and Williamson counties. However, additional infestations are suspected beyond these areas of infestation. Currently, the little that is known regarding the biology, behavior, and

management of this invasive ant species is posted on the Center for Urban and Structural Entomology website (Drees 2012).

Nylanderia fulva workers, are tawny in color, monomorphic, ~2 mm in length, have long legs and antennae (12-segmented with no club), and their bodies have numerous, long, coarse macrosetae (Meyers and Gold 2008). Males are approximately the same size and color as workers, and can be distinguished from males of *N. pubens* by the reduced number of macrosetae originating from the triangular shaped, less sclerotized parameres (Gotzek et al. 2012). Queens are much larger than workers and notably darker brown in color.

Nylanderia fulva has large colonies containing many queens, workers, and brood (eggs, larvae, and pupae). Workers exhibit loose foraging trails and individual ants forage erratically, hence the typical reference to "crazy" ant. The size of the infestations can be large; infesting entire subdivisions (Deer Park, TX), industrial sites (Texas City, TX), agricultural operations (East Columbia, TX), and honeybee apiaries (Pearland, TX). *Nylanderia fulva* colonies can be found under or within almost any object or void, such as stumps, soil, concrete, rocks, potted plants, and many objects that retain moisture. Nests primarily occur outdoors, but worker ants will forage indoors, into homes and other structures.

Behaviorally, *N. fulva* is similar to *L. humile*. Both species prefer mesic over xeric scrub fragments, evidenced by their ability to penetrate further, and attain higher abundance in forested or heavily shaded areas rather than unshaded lawns or grasslands (Holway et al. 2002a). Like *L. humile*, *N. fulva* routinely move their nests closer to

resources and to more abiotically suitable sites (Tsutsui and Suarez 2003). Horton (1918) reported that from a 19 acre orange grove in Louisiana, 1.3 million *L. humile* queens and 1000 gallons of workers and brood were collected in a span of 1 year (Tsutsui and Suarez 2003). It is not uncommon to find 15-20 *N. fulva* queens underneath each landscape object in Texas infestations.

Field observations suggest that during the spring, *N. fulva* population densities begin to increase with a peak around mid-summer. Population densities remain high through fall, but seem to decrease dramatically in winter months. Although colonies periodically produce winged male and female alates, no mating (nuptial) flights have been observed in the field.

Nylanderia fulva derive nutrients from a variety of sources. *Nylanderia* sp. workers commonly "tend" hemipterous insects such as aphids, scale insects, whiteflies, planthoppers (Humphreys 1998) and mealybugs (Carver et. al. 1987), for their carbohydrate rich exudate (honeydew). *Nylanderia fulva* workers are attracted to sweet parts of plants including floral and extrafloral nectaries and over-ripe fruit. Worker ants also kill and scavenge other insects and small vertebrates for protein.

Nylanderia fulva is a semi-tropical ant species. As a result, population densities will likely be limited by cooler weather conditions (i.e., freezing winters) and decreased humidity levels (Holway 1998), and will likely infest much of the Gulf Coast of the United States (Gotzek et al. 2012). However, with the recent focus of research on the effects of climate change, it is predicted that species will respond by shifting their current ranges to occupy their climatic niche (Bellard et al. 2012). For example, *L.*

humile is predicted to retract its range in tropical regions, and expand its range in higher latitudes (Roura-Pascual et al. 2004).

The projected range limit of ants, particularly *S. invicta*, have been assessed and revised several times. Most predictors expect that climate, specifically cold and drought tolerance, is the limiting factor for *S. invicta* expansion, but these predictions generally over-exaggerated their potential range (Tschinkel 2006). Korzukhin et al. (2001) utilized a model that incorporated colony size, growth rate, worker longevity, temperature-tracking behavior, cold coma, freeze kill of workers, and temperature thresholds for brood production (Tschinkel 2006). Results showed that *S. invicta* has the potential to inhabit the southern U.S. including regions as far west as Texas, except for the arid western portion and the panhandle, as far north as 80 to 150 km into Arkansas, Oklahoma, Tennessee and Virginia, and the wetter portions of California. Unfortunately, many of the parameters used in the Korzukhin model are unknown for *N. fulva*.

There are three mechanisms for ant populations to expand their range: human mediated jump dispersal, natural jump dispersal through mating flights, and spread through colony budding (the division of colonies into sub-colonies that disperse to extra nest sites) (Holdobler and Wilson 1990). For ant species that exhibit budding behavior such as *N. fulva*, human-mediated dispersal is their only means of quickly traveling long distances, which can make it difficult to predict long distance invasion rates (Holway and Suarez 1999). *Linepithema humile* has been shown to have a maximum rate of spread through budding of a few hundred meters per year (Holway and Suarez 1999, Suarez et al. 2001), and *N. fulva* was shown to advance 100 m per month with rivers

serving as the only barrier to impede migration in Columbia (Zenner de Polania 1990). In Texas, *N. fulva* colonies move at a reported rate of ~20 and ~30 m per month for a neighborhood and industrial area, respectively, but are predicted to exhibit greater expansion rates in rural landscapes (Meyers 2008).

Pest Status of *N. fulva*

Nylanderia fulva demonstrate synanthropic associations with humans, which is characteristic of tramp species, and therefore provide an assortment of problems in homes, businesses, and agricultural settings. In areas infested with this species, large numbers of ants have accumulated in electrical equipment, causing short circuits and clogging switching mechanisms resulting in equipment failure (Meyers 2008). This is a result of the fact that some ants are strongly attracted to both AC and DC electric fields; however, *S. invicta* have been shown not to be attracted to ozone, magnetic fields or electromagnetic fields (MacKay et al. 1992). Shorting of electrical equipment by ants can also be a result of opportunistic foraging and harborage scouting rather than an attraction to electricity. Worker ants are most likely attracted to the alarm pheromone released when a single ant interrupts the electrical contact and is electrocuted (Drees 1998).

In infested sites around the Houston area, large numbers of *N. fulva* have caused great annoyance to residents and businesses. They can occur at such high densities that the ground and trees can be covered with ants. In some situations, the extreme population densities have prevented residents and their companion animals from

enjoying their time outdoors. Although these ants do not have a sting, worker ants possess an acidopore that they use to project formic acid for defense. They are capable of biting, yet the bite is weak and quickly fades. Wildlife, such as ground nesting birds, will likely be affected due to the irritation caused by so many ants in the landscape.

Field observations suggest that *N. fulva* displace RIFA and most other ant species in areas of heavy infestation through exploitative and interference competition. *Nylanderia fulva* has reportedly also displaced ant species in South America (Zenner de Polania 1990, Harris 2002, Davis et al. 2008). Similar to *L. humile* in California, the competitive mechanisms and behaviors of *N. fulva* that allow for the displacement of other ant species include: the use of chemical defensive compounds, physical aggression by workers, initiation of interspecific interactions and nest raiding more often than native ants, preying upon foundress queens, acquiring food sources aggressively and quickly in high numbers, remaining at food sources longer than native ants, omnivorous feeding habits, being active through all seasons, diel foraging activity, and adjusting foraging behavior to local worker density (Holway et al. 2002b).

Management of *N. fulva*

It is apparent that *N. fulva* have surpassed the prevention level of management in Texas, and containment appears daunting if not impossible. Simberloff (2003a) suggests that the need for intensive population biological research is limited relative to the need for early detection and expedient chemical and mechanical control. Furthermore, the preamble to the Convention of Biological Diversity states that "where there is a threat of

significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat" (Simberloff 2003b).

Unfortunately, *N. fulva* workers are not attracted to most ant bait products, but the one known granular product to which they are attracted (Whitmire Advance® Carpenter Ant Bait formulation containing abamectin) may not offer enough control at the label rate or be effective as a stand-alone treatment. Furthermore, since Advance® Carpenter Ant Bait (ACAB) is only labeled for residential areas, there is currently no attractive bait available for wildlife breeding areas or agricultural sites. Research is required before cost-effective and environmentally sound management practices can be achievable.

There are only a few treatments available for *N. fulva* in urban areas that offer temporary buffer zones around structures using contact insecticides applied to surfaces. These include exterior contact sprays containing fipronil such as Termidor®SC (Meyers 2008) and Taurus®SC. An extended label for Termidor® allows for this product to be applied to the outside of structures 0.91 m up and 3 m out (Meyers 2008). Top Choice™ and Taurus®G are granular fipronil products that can be broadcast in residential landscapes and lawns, but may only be used once per year. Phantom® (chlorfenapyr) is recommended for indoor use as a standard crack and crevice application (Meyers 2008). Talstar® (bifenthrin) can be used as a drench for potting media to prevent *N. fulva* from infesting plant nursery stock. In food crops, it is recommended that applications of spinosad, a certified "organic" product (Organic Materials Review Institute), be utilized. These products, used in conjunction with ACAB, have achieved some level of

management according to pest control operators (Tom Rasberry 2011, personal communication).

Effective products for the management of *N. fulva* are not typically available to retail consumers lacking a pest control operator's license, and a professional pest control provider should be contacted in most cases. These licensed pest management professionals are trained and experienced, and will offer alternative treatments that are unavailable to unlicensed consumers. After a treatment, or when making multiple applications over time, piles of dead ants must be removed from treated areas in order to retreat surfaces underneath and allow for ants contact to treated surfaces.

Immediate attention should be focused on research involving integrated pest management (IPM) strategies for *N. fulva*. Eradication of established populations has not been feasible, but an integrated approach to management will allow for complementary strategies and techniques to be used simultaneously. Prevention of multiple introductions and human-mediated jump dispersal should be the cornerstone for *N. fulva* IPM. Cultural practices may have to be modified to prevent heavy infestations. For example, it has been suggested that a cultural control strategy for *L. humile* might be to reduce urban irrigation in order to maintain functionally diverse communities of native ants (Holway and Suarez 2006). Development of bait matrices tailored for *N. fulva* nutritional needs, coupled with effective active ingredients such as insect growth regulators, would also be paramount to the success of *N. fulva* management. Finally, exploration of *N. fulva* natural enemies may lead to an introduction of biological control agents.

Priority should be given to the immediate eradication on newly established populations (Tsutsui and Suarez 2003). However, the delayed detection of invading species for long periods of time allows for population densities to reach intensities that confound management strategies. Therefore, a policy of vigilance and prevention of invaders is ultimately recommended rather than a policy of containment and control (Tsutsui and Suarez 2003).

The goal of these works is better understand the biological idiosyncrasies of this species that may ultimately lead to the mitigation of the invasive success of *N. fulva*. This dissertation addresses biological, behavioral, management, and collection techniques of *N. fulva*. Specifically, topics investigated include a modified drip technique for extracting ants from their substrate, the effectiveness of a carpenter ant bait as a stand-alone treatment, the foraging preference and peak activity of workers, the reproductive potential of queens, and the ability of this species to translocate pathogenic microorganisms.

CHAPTER II

A MODIFIED DRIP TECHNIQUE FOR EXTRACTING *Nylanderia fulva* (HYMENOPTERA: FORMICIDAE) FROM SUBSTRATES

Introduction

Laboratory studies involving ant species often require ants to be separated from nesting material. Techniques for extracting ants include the drip technique for *Solenopsis invicta* (Banks et al. 1981), aspiration (Solis et al. 2007), sifting substrates through sieves, and drying or heating the substrate to force the emigration of ants (Markin 1968). Regarding *Nylanderia fulva* specifically, there existed a need for the development of a more efficient method of non-floating ant extraction that allows for all castes and brood to be recovered. A modified drip technique utilized for multiple tramp ant species is herein described.

This technique was initially developed for *N. fulva*, an introduced tramp ant species originally described from Brazil (Zenner de Polania 1990). As is typical of its congeners, *N. fulva* do not construct elaborate nests. Instead, they take advantage of ephemeral nesting sites such as landscape objects, preformed cavities, and almost any empty space as long as it provides adequate moisture and warm temperatures (Zenner de Polania 1990). They do not exhibit rafting behavior as do *S. invicta* and, therefore, the drip technique cannot be used to extract their colonies. In their introduced range, *N. fulva* exhibit extremely high population densities, and can relocate colonies quickly

when disturbed. The modified drip technique takes advantage of this behavior by forcing the colony to relocate to dry ground during flooding.

Aguillard et al. (2011) independently arrived at a similar technique for extracting *N. fulva*. Their technique involves the collection of substrate into 55 L storage containers with the top 7 - 10 cm coated with a fluoropolymer. The substrate is held down using wire mesh and a dish is provided for the immigration of ants. Flooding then commences by initially adding 500 ml of water, waiting 30 minutes, and then adding 1 L of water every 30 minutes until the substrate is covered. This technique results in the separation of all castes and brood from nesting substrates. However, utilization of 55 L storage containers is cumbersome and fluoropolymers tends to be untidy, staining any fabric. Two working days would be spent extracting a colony from its substrate using Aguillard's technique. The following technique provides a more time efficient, and passive way of extracting whole colonies that is easier to perform, lighter in weight, and will take up less laboratory space per colony.

Materials and Methods

Ants utilize a variety of substrates for colony (nesting sites including workers, alates, and brood) establishment including soil, leaf litter, logs, cavities in trees, and just about any crack or crevice. In the case of *N. fulva*, it appears that colonies in the field are a subset of a supercolony. Once colonies were located, the substrate in which the colony was using for refuge was collected using a shovel and placed into an 18.9 L bucket. Buckets were not filled more than half way with substrate to allow room for

dripping procedures. Prior to collection, the interior walls of the buckets were coated with baby powder (talc) to prevent the ants from escaping (Fig.1) (Bomford and Vernon 2005, Drees 2002). Once the collected colonies were transported back to the laboratory, the substrate was forced to settle by gently shaking the bucket so that the top of the substrate created a level surface. The colonies were then allowed to settle for 12 – 24 hours (hereafter referred to as the latent period) to allow ants to create tunnels throughout the disturbed substrate.

Artificial harborage, consisting of a tower made from layers of cardboard with additional cardboard spacers, was constructed. Shelves of the tower consisted of 10.2 cm square pieces of cardboard (Fig.2). Cardboard thickness for shelves was small enough to deter ants from using the corrugation as refuge (≤1.5 mm for *N. fulva*). Two 7.6 cm x 1 cm strips of cardboard were cut to serve as spacers between shelves. Cardboard thickness for spacers was large enough to allow for workers and queens to move freely between them (4 mm for *N. fulva*). The spacers were adhered to one side of a shelf using non-toxic adhesive, and allowed to dry (Fig.2). The side used to attach the spacers was considered the bottom of the shelf. Once all of the spacers were attached and dried, a tower was constructed by stacking six shelves on top of each other, bottoms down (Fig. 3). Note that the bottom shelf of the tower did not have spacers, which prevented ants from lodging underneath the tower while dripping.

Following the latent period, a stone, tile, or alternative weight was placed on top of the substrate to prevent floatation which could be used by ants as a raft and provide a foundation upon which the tower rested (Fig. 4). Next, a bridge was constructed out of a

Fig. 1. An 18.9 L bucket dusted with talcum powder to prevent ants from escaping.

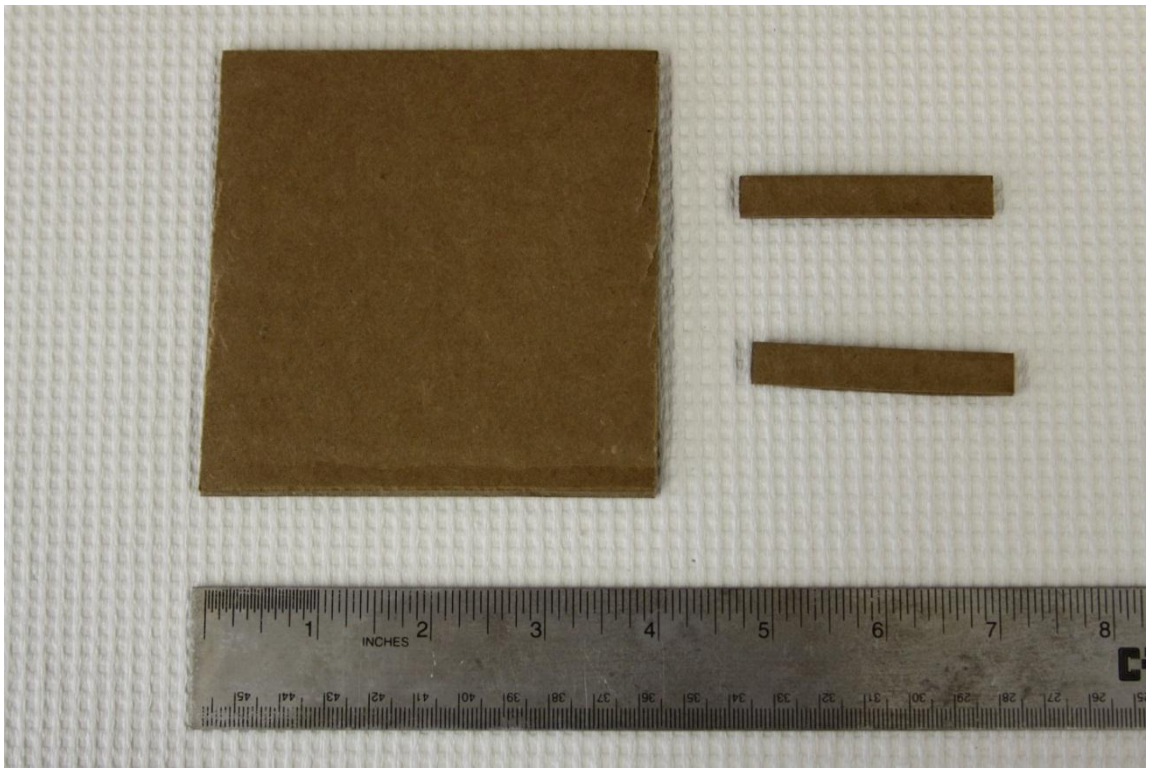


Fig. 2. A shelf and spacers for the tower with a ruler for a scale reference.

Fig. 3. A cardboard tower constructed by stacking 6 shelves on top of each other, bottoms down.

Fig. 4. A weight placed on top of the substrate to prevent floatation, which can be used by ants as a raft, and to provide a foundation upon which the tower rested.

28 cm piece of stiff wire mesh (6 -12 mm openings are recommended). Two opposite ends of the wire were bent downward at a 90° angle, 8 cm from each end, to create a bridge shape (Fig. 5). The bridge was then placed on top of the weight in the bucket, and the cardboard tower was placed on top of the bridge (Fig.6). The bucket was placed underneath a sink faucet and turned on to a slow drip (about 1-2 drops per second) (Fig 7). The water was allowed to rise to just below the tower, which took 4-8 hours depending on the amount of substrate collected and the actual rate of water flow. As the bucket was flooded, worker ants transported brood and queens into the tower. The tower was then removed from the bucket and placed into a nesting chamber.

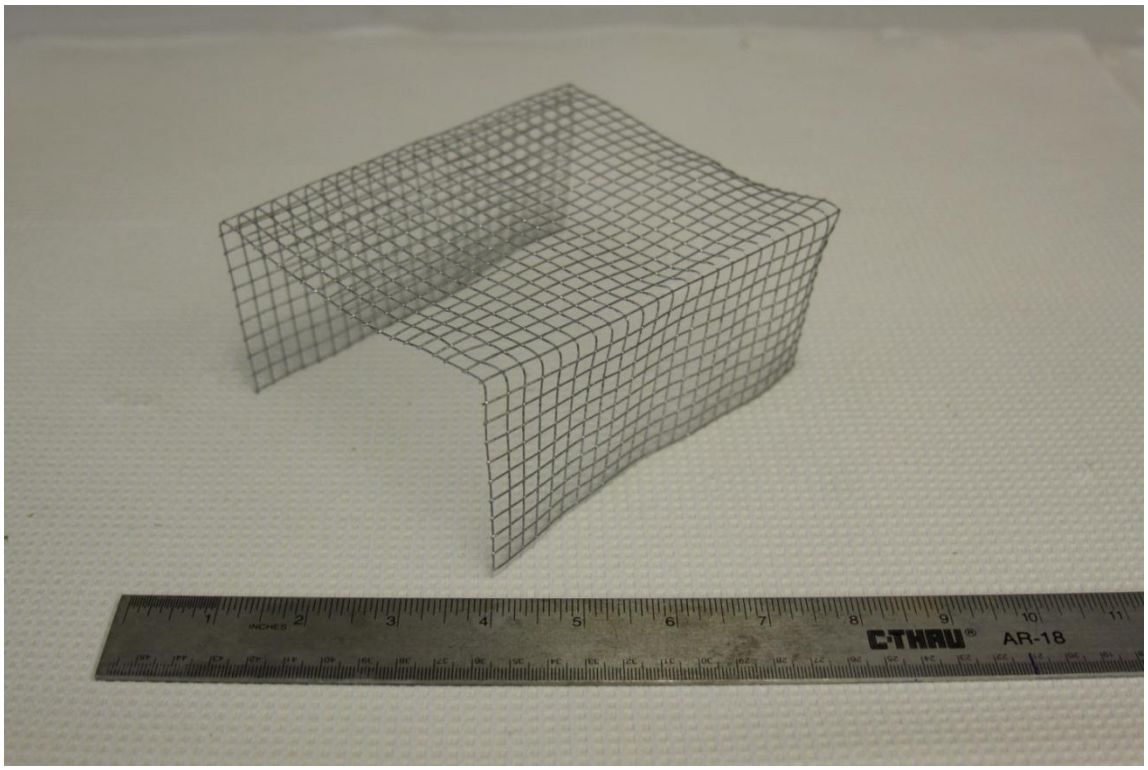


Fig. 5. A bridge constructed from a 28 cm piece of stiff wire mesh with 6 mm openings. Two opposite ends of the wire bridge were bent downward at a 90° angle, 8 cm from each end, to create a bridge shape.

Fig. 6. The final construction consisting of a bucket with substrate and ants, a weight, bridge, and cardboard tower.

Fig. 7. A drip rate of 1-2 drops per second was used to allow ants to move brood and queens into the cardboard tower.

A plastic container with vertical sides coated with a fluoropolymer was used for maintaining colonies in the laboratory. The nesting chamber was provisioned with harborage, water, 10% sucrose water as a carbohydrate source, and dead insects such as crickets and termites as a protein source.

Harborage consisted of either moistened plaster of Paris filled petri dishes (Banks et al. 1981), or half-filled vials of water, plugged with a cotton stoppers (Solis et al. 2008), with a ~3 mm layer of plaster of Paris on top of the cotton, and vials were wrapped in aluminum foil to provide a dark, humid microenvironment. Colonies were kept in an environmental chamber set at 27°C, 80% RH, and a 10:14 light to dark photoperiod.

Results and Conclusions

The modified drip technique allows for the separation of colonies of non-floating ants from substrates and benefits include its ease of use, practicality for laboratory and field application, suitability for various species, time efficiency, and reusable components. As long as the substrate was able to be collected in an 18.9 L bucket and transported to a faucet, this technique worked for many species.

Baby powder was preferred over fluoropolymers due to the ease of reuse of talc coated buckets and the propensity of fluoropolymers to rub off on clothing. One colony per bucket is recommended to prevent intercolony aggression. An alternative method for smoothing out the soil surface prior to the latent period is to use your hand or other instrument. The number of shelves per tower will depend on the size of the colony being

extracted, with 6-7 layers typically used for one large colony of *N. fulva*. After flooding, layers of the tower can be placed into separate nesting chambers if splitting colonies is desirable. Artificial diets are also a good alternative to the provisions mentioned, and may be necessary for some species.

This technique has proven effective for *N. fulva*, *Linepithema humile*, and *Paratrechina longicornis*, but not *Brachymyrmex* sp. It appears that *Brachymyrmex* sp. were not capable of moving their brood rapidly enough to avoid drowning, and their attempts to move the colony was minimal.

CHAPTER III

EFFECTS OF ADVANCE® CARPENTER ANT BAIT ON *Nylanderia fulva* (HYMENOPTERA: FORMICIDAE) POPULATION DENSITY IN EAST COLUMBIA, TEXAS

Introduction

Since 2002, populations of *N. fulva* have been found in localized 'spot' infestations in 24 Texas counties (Fig. 8). New infestations are suspected beyond those already reported. This species has the potential to spread well beyond its current, known range in Texas. *Nylanderia fulva* appears to be a tropical or semi-tropical ant. Thus, population expansion will likely be limited to the southern region of Texas and the U.S. by cooler weather conditions and decreased humidity in northern climates (Holway 1999).

Nylanderia fulva is a tramp ant species, polygynous, unicolonial, appears to reproduce by colony budding, and largely dispersed by human activity (Hölldobler and Wilson 1990, Passera 1994). In addition to human aided dispersal, colonies spread naturally by ground migration at a reported rate of ~20 and ~30 m per month in neighborhoods and industrial areas, respectively (Meyers 2008). However, colony expansion is predicted to be greater in rural landscapes than in urban neighborhoods and industrial areas (Meyers and Gold 2008).

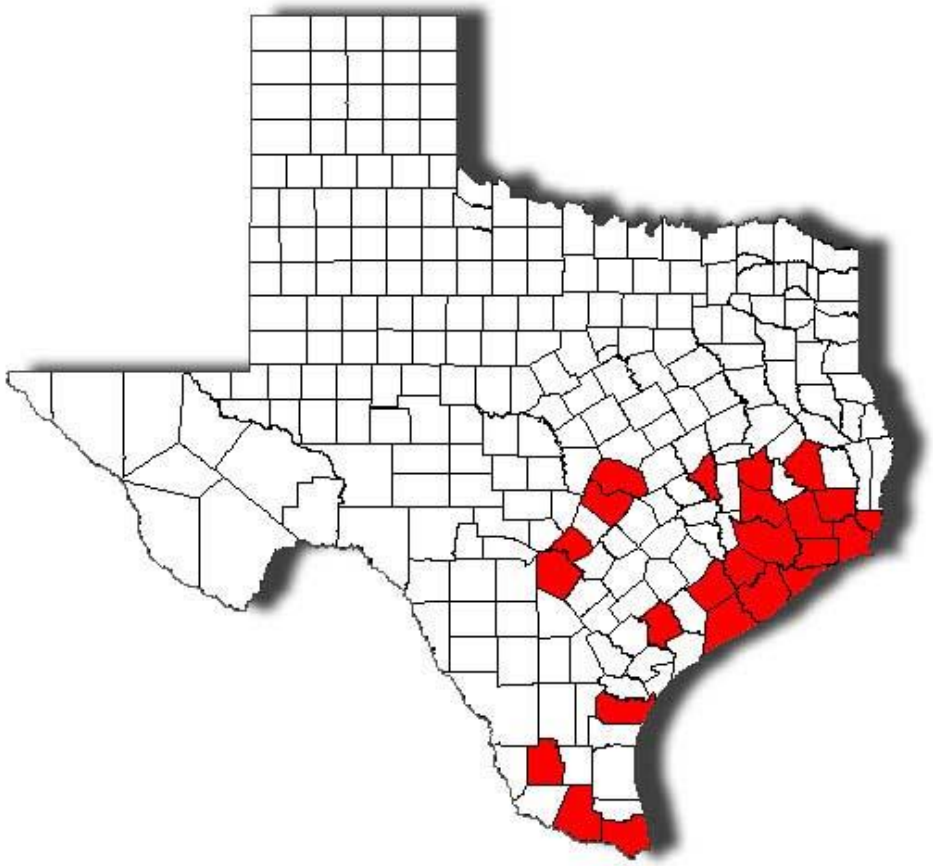


Fig. 8. The 24 *N. fulva* infested Texas counties: Bexar, Brazoria, Brazos, Cameron, Chambers, Comal, Fort Bend, Galveston, Hardin, Harris, Hidalgo, Jefferson, Jim Hogg, Liberty, Matagorda, Montgomery, Nueces, Orange, Polk, Travis, Victoria, Walker, Wharton and Williamson counties (Drees 2012).

The size of *N. fulva* infestations can be large, infesting entire subdivisions (Deer Park, TX), industrial sites (Texas City, TX), and agricultural operations (East Columbia, TX). *Nylanderia fulva* colonies nest in and under a variety of landscape articles, such as stumps, soil, concrete, rocks, and potted plants. Nests primarily occur outdoors, but worker ants regularly forage indoors, especially during winter months.

Adult colony members, including queens, males and workers are reddish-brown, although variations in body color have been observed. Worker ants are monomorphic, 2 mm in length, have 12-segmented antennae with no club, and their bodies have numerous, long, coarse hairs (Meyers and Gold 2008). A major identifying characteristic is 2-3 pairs of macro setae on the mesonotum. Workers exhibit loose foraging trails and individual ants also forage erratically, hence the typical reference to "crazy" ant.

The characterization of the pest status of *N. fulva* requires the consideration of economic, ecological, and social aspects related to infestations. In areas infested by *N. fulva*, large numbers of ants accumulate in electrical equipment, causing short circuits resulting in equipment failure (Meyers 2008). *Nylanderia fulva* appeasr to displace red imported fire ants (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in areas of heavy infestation. Being a member of the subfamily Formicinae, *N. fulva* does not possess a sting like *S. invicta* (subfamily Myrmicinae), but use a formic acid projecting acidopore and biting for defense. *Nylanderia fulva*, has been a serious pest in South America and is reported to displace all other ant species within its range (Zenner de Polania 1990, Harris 2002, Davis et al. 2008). In addition, *N. fulva* caused chickens to die of asphyxia due to ants obstructing nasal passages. Larger animals, such as cattle, have been observed with large numbers of these ants around the eyes, nasal area, and hooves. In apiaries around Houston, Texas *N. fulva* have caused colonies to abscond, but no direct predation has been observed (pers. com. Brad Metz). Wildlife, such as

ground nesting birds, will likely be affected due to irritation and possible pipping of eggs.

In Colombia, Zenner de Polania (1982, 1990) attempted many control tactics against *N. fulva* including exclusion, cultural control, toxic baits, and contact insecticides. Exclusion using 20 cm wide water moats, BIOTAC 3, and BIOTAC 19 sticky bands around tree trunks was efficient and economical but was not readily accepted by farmers. Cultural control including the removal of potential artificial harborage from around houses, nurseries, and plantations decreased permanent nests by 23% but did not keep crazy ants at sub-economic levels. It was noted that it is necessary to combine cultural control with effective chemical control methods. Toxic baits evaluated, reportedly included various carriers and attractants combined with lindane, aldrin or carbaryl. It was determined that a matrix of corn bran, fish meal, sugar solution, propionic acid, and pork lard with carbaryl (a carbamate) as the active ingredient provided 2 months of control.

The use of bait formulated insecticides is common for ant management, such as with RIFA, due to ecological and economic advantages based on reduced effects to non-target organisms, specificity of the diet preference of target organisms, and their effectiveness of reaching queens and larvae (Banks 1990, Zenner de Polania 1982, 1990). Insecticide bait formulations contain highly attractive food materials laced with delayed-action insecticides. They are arguably the most efficacious methodology for controlling structure-infesting ant species (Banks 1990, Bueno and Campos-Farinha 1999, Gusmao et al. 2011, Klotz et al. 1997, Lopez et al. 2000, Stanley and Robinson 2007).

Unfortunately, *N. fulva* workers, much like other crazy ant species (Stanley and Robinson 2007), are not attracted to corn grit with vegetable oil-based bait products traditionally tailored to *S. invicta* preference (Drees et al. 2010a). Whitmire Advance® Carpenter Ant Bait (ACAB) formulation containing abamectin, is the only commercial bait known to be attractive to *N. fulva*. Abamectin blocks gamma-aminobutyric acid (GABA) receptors used to transfer chloride ions to the receptors of muscles, causing cessation of foraging and egg laying, paralysis, and death of individuals within 3-4 days (Ware and Whitacre 2004). Avermectins (the insecticide class containing abamectin) have been shown to be effective against *Linepithema humile* (Baker et al. 1985), *Lasius neoniger* (Lopez et al. 2000), and *Solenopsis invicta* (Glancy et al. 1982). However, Hooper Bui and Rust (2000) found that while *L. humile* populations were suppressed, workers and queens survived treatments with abamectin and suggested that the remaining population may again become pestiferous. Unfortunately, ACAB is only labeled for 0.184 kg/ha in residential areas, and no currently labeled bait products are registered for agricultural sites. This 0.184 kg/ha rate may prove to be insufficient for managing large densities of some ant species like *L. humile*, especially those surrounded by areas not permissible to treat because of label restrictions.

In Columbia, Zenner de Polania (1990) evaluated contact insecticides for immediate knockdown effects and long term residual effects against *N. fulva* around houses, cattle ranges, and coffee plantations. Contact insecticides provide quick mortality of worker ants and are often preferred by homeowners for this reason. In Zenner de Polania's study, chlorpyrifos provided the highest efficacy for 43 days after application. Finally,

Zenner de Polania (1990) concluded that no single control method for *N. fulva* would provide acceptable population management and that an integrated management approach was necessary. Scheffrahn and Warner (2011) achieved ~6 weeks of control of *N. pubens* (possibly *N. fulva*) when using Talstar XTRA GC (bifenthrin and zeta-cypermethrin) and Transport GHP (acetamiprid and bifenthrin) on vegetation around, and directly on golf cart paths in South Florida.

Many of the typical control tactics for other ant species do not provide adequate control of *N. fulva*. Colonies predominantly nest outdoors, so reliance on indoor control methods alone has not proven effective. However, there are long-residual, non-repellent contact insecticide treatments available for urban areas that offer temporary "buffer zones" in and around structures. These products include: Phantom®SC (chlorfenpyr) as a crack and crevice spray inside structures, Termidor®SC or Taurus®SC (fipronil) as a perimeter spray around structures, and TopChoice® or Taurus®G (fipronil) as a broadcast, granular, contact insecticide. Although TopChoice® and Taurus®G are not yet labeled specifically for *N. fulva*, the Federal Insecticide Fungicide and Rodenticide Act (FIFRA, section 2ee) states that "by law, a pesticide can only be applied to a site that is identified on the label, even though specific pests may not be indicated." (Staples 2012). Fipronil is an effective barrier treatment to control ants such as *L. humile* as a result of its delayed toxicity (270-960 min) allowing for a greater percentage of foragers to be exposed after recruitment, with subsequent transferability to non-exposed workers (Rust et al. 2004, Soeprono and Rust 2004a,b). Termidor®SC has been issued a Section 18 quarantine exemption label by the Texas Department of Agriculture for the 24 Texas

counties known to have *N. fulva* infestations. This exemption allows the product to be used 0.91 m up and 3.08 m out from structures as a direct result of research by Meyers (2008). Used in conjunction with ACAB, these products have achieved ~1 month of significant *N. fulva* management (Meyers 2008), as compared to untreated control plots. Therefore, this is the current recommended management plan for *N. fulva* around homes and structures in large landscape areas. A community wide treatment of entire populations with these types of products could provide the greatest chance of spot eradication (Sheffrahn and Warner 2011).

The experiment reported herein used ACAB as a stand-alone treatment for *N. fulva*. GIS interpolations were utilized to depict *N. fulva* relative abundance throughout treated and non-treated areas, and when viewed sequentially, depict achieved control and subsequent rebound of *N. fulva* populations.

Materials and Methods

In 2008, a new site of *N. fulva* infestation in East Colombia (Brazoria County), TX was discovered by Corrie Bowen (County Extension Agent, Texas A&M AgriLife Extension) and Paul Nester (Extension Program Specialist, Texas A&M AgriLife Extension). This area is comprised of ~40.5 ha including roughly 8 ha of residential properties, and 32.5 ha of mixed woodlands and pasturelands used for hay production, storage and cattle foraging. The climate in East Columbia is subtropical. The boundaries of the infestation were identified and recorded via global positioning system (GPS) every August from 2008-2011. As a result of our knowledge of the population extent

and home owner cooperation, this site was found to be ideal to conduct preliminary research on the basic biology, seasonal population dynamics, impact on wildlife and agriculture, and demonstration of current broadcast bait treatment effectiveness.

This isolated infestation provided an opportunity to test a 0.184 kg/ha rate of Advance® Carpenter Ant Bait (ACAB) as a stand-alone treatment for *N. fulva*. *Nylanderia fulva* ubiquitously infests the site, which is bisected by a single road (Fig. 9). The test site extended 2.4 km from a large property at the southwestern end of the road to a church located at the northeastern part of the community. A power-line clear-cut area occurred approximately 460 m perpendicular to the single road. Three treatments with ACAB were administered throughout 2009 (May 6th, May 26th, and Sept. 18th). Pretreatment relative abundance measurements of the *N. fulva* infestation were taken once a week for 3 weeks prior to treatment to assess changes in population levels using the methods described below.

ACAB was broadcast using a Herd seeder (model GT-77 ATV; Herd Seeder, Longansport, IN) mounted on all-terrain vehicles. The Herd spreaders were calibrated to broadcast ACAB at a rate of 0.184 kg/hec.

Food lures were used to assess relative abundance of *N. fulva* and the presence of other ant species foragers by determining the number of ants that were collected with a 4 mm slice of hot dog (Bar S Hot Dog) after 60 min of exposure. The hot dog slices were placed on the ground with a flag skewered through the middle for ease of location/collection. Foraging is a temperature-limited function (Tschinkel 2006).

Fig. 9. Survey of *N. fulva* population extent from 2008-2011. The average yearly distance of population extent movement was ~207.4 m/yr. The 2011 *N. fulva* population encompassed 276.5 ha.

Therefore, shaded areas were utilized for food lure placement to avoid error in the lack of presence of *N. fulva* due to extreme heat. If no natural shade was available, a 15.2 cm paper plate was used to shade the hot dog. This method has been used to assess RIFA populations and has been suggested for use with *N. fulva* (Calixto 2009, personal communication).

Transects were established to monitor *N. fulva* relative abundance throughout the study using food lure stations (Bar S Hot Dog slices). These efforts also monitored other ant species which empirical observations showed were negatively correlated with *N. fulva* density. Transects with monitoring stations at regular intervals of 30.48 m were established: 1) through residential properties from within the infested area at the southwestern most property to 304.8 m beyond the edge of the current infestation to document the effects of broadcast bait treatment, as well as population expansion from the edge of the known infestation; and, 2) through the power-line clearing perpendicular to the Brazos river to monitor ant foraging activity in a cattle grazing and hay pasture (Fig. 10). The power-line clearing and several properties throughout the community were used as untreated control sites. *Nylanderia fulva* abundance observations were taken once per week, as weather and circumstances permitted.

Using relative abundance of *N. fulva* to determine population affects by ACAB, ant counts from treated areas were compared to counts from untreated areas. Each observation date was analyzed using Student's t-Test to determine significance between treated and untreated plots (SPSS 2007). Effects of ACAB on *N. fulva* populations were established by assessing significant differences between treated and untreated plots and by utilization of the Henderson-Tilton formula. Henderson-Tilton's formula was used to calculate corrected efficacy percentages in pesticide trials to compensate for differences in control plot populations before and after treatment (Henderson and Tilton 1955).

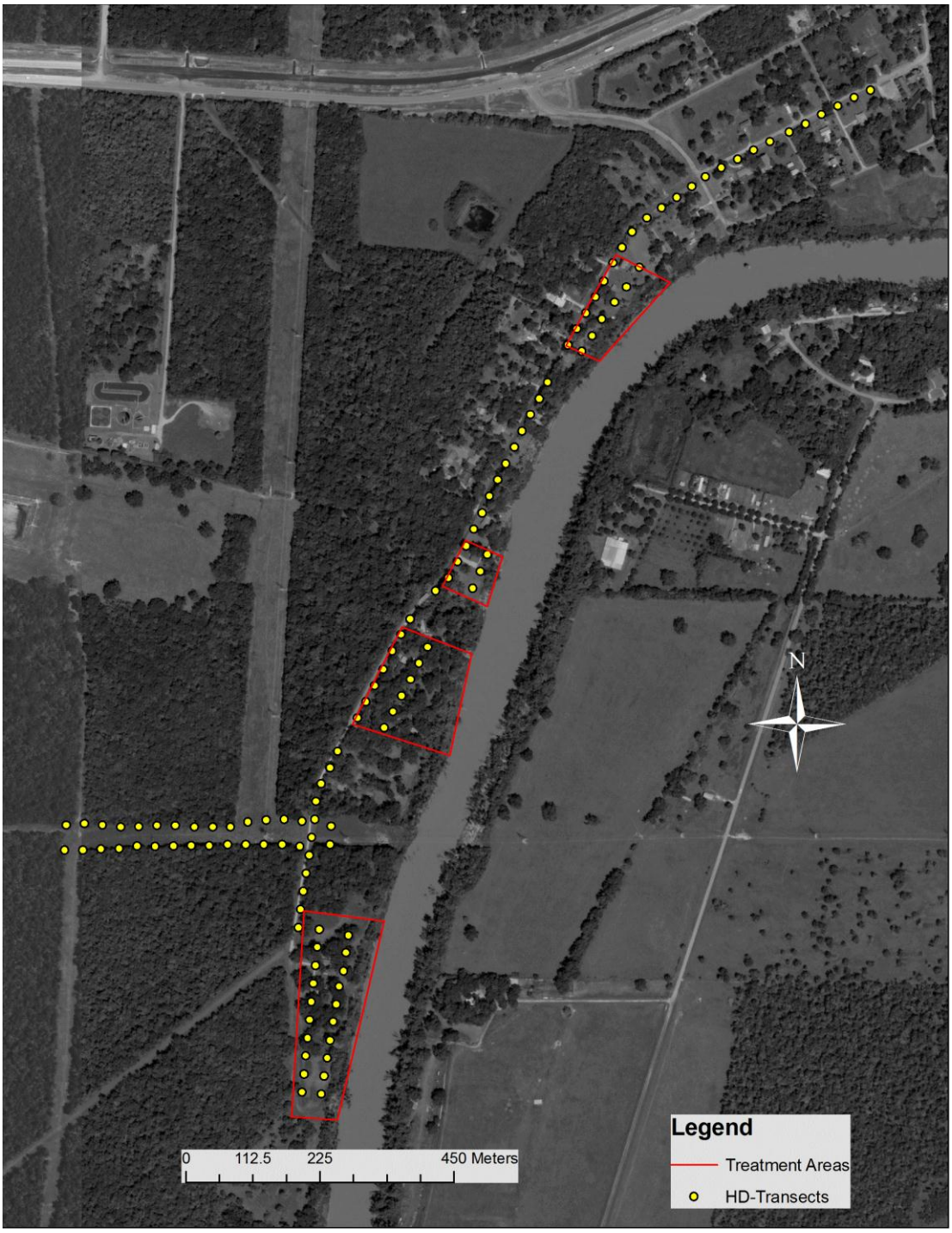


Fig. 10. Food lure transects and treatment areas in East Columbia, TX

Two orthophotos were downloaded from the Texas Natural Resources Information System website, following the standard USGS Quarter Quad Grid, of Brazoria County to serve as the background for interpolation maps. Two photos were needed to cover the entire East Columbia community. The cell size was .5m x .5m and were projected using NAD, 1983, UTM, Zone, 15N. All maps and features hereafter used the same cell size and projection.

GPS coordinates of food lure locations were exported from Google Earth using DNR Garmin software. These coordinates were used to depict the XY coordinates of the observation transects used for this study. The table was then exported to make it an editable file and served as a master observation transect table. Excel spreadsheet data of relative abundance of ants from each collection date were saved as comma delimited files (CSV). These CSVs were imported into ArcMap. Tables from each observation date were then merged with the transect table and exported to convert them to editable files. This resulted in ArcMap data tables with XY coordinates that corresponded to *N. fulva* populations.

In ArcMap, a buffer of the hot dog food lure transects was created, extending 15.24 m past any given observation point. A buffer distance of 15.24 m was chosen based upon half the distance of observation points from one another. This polygon was used as a mask for future interpolations to represent the extent of the observation area.

Four polygon shape files were created in ArcMap and grouped to serve as the extent of the treatment plots. The drawing was then exported as a permanent feature. The interior was made hollow, and the border made red.

Data sets were analyzed using the inverse distance weighted (IDW) tool with the following parameters: Input - Date of Interest; Z value - *N. fulva* population; Power - 2; Search Radius Type - Variable; Number of Points - 12; Output Cell Size - 0.5m. The default classification setting of 9 was used. The resulting IDWs were then exported and converted to permanent features. The stretched color scheme was utilized using the "brown to blue-green divergent, bright" color.

Results

The yearly survey of *N. fulva* population extent revealed an annual population spread of ~207.4 m/yr. (Fig. 9). The population was ubiquitous and was hypothesized to be one large supercolony that covered 276.5 ha by August of 2011.

There was no statistical difference in *N. fulva* populations between control and treatment plots (Fig. 11 and 12a, Table 1,). Ants were uniformly distributed throughout the study area. There was a significant difference ($p < 0.001$) between treatment and controls 5 days after the initial treatment, which resulted in a 58% reduction in *N. fulva* (Fig. 12b). After 13 days there was only a 37% difference ($p < 0.001$) between controls and treatments (Fig.12c). At 20 days post treatment, there was no significant difference between controls and treatments ($p = 0.897$) (Fig. 12d). Therefore, a second treatment was administered on that same date. Six days after the second treatment there was a 77% reduction ($p < 0.001$) in the *N. fulva* population (Fig. 13a), and after 13 days there was 52% reduction ($p = 0.001$) (Fig. 13b). Twenty days later, there was still a significant 51% reduction in the population ($p = 0.036$) (Fig. 13c). At 27 days there was

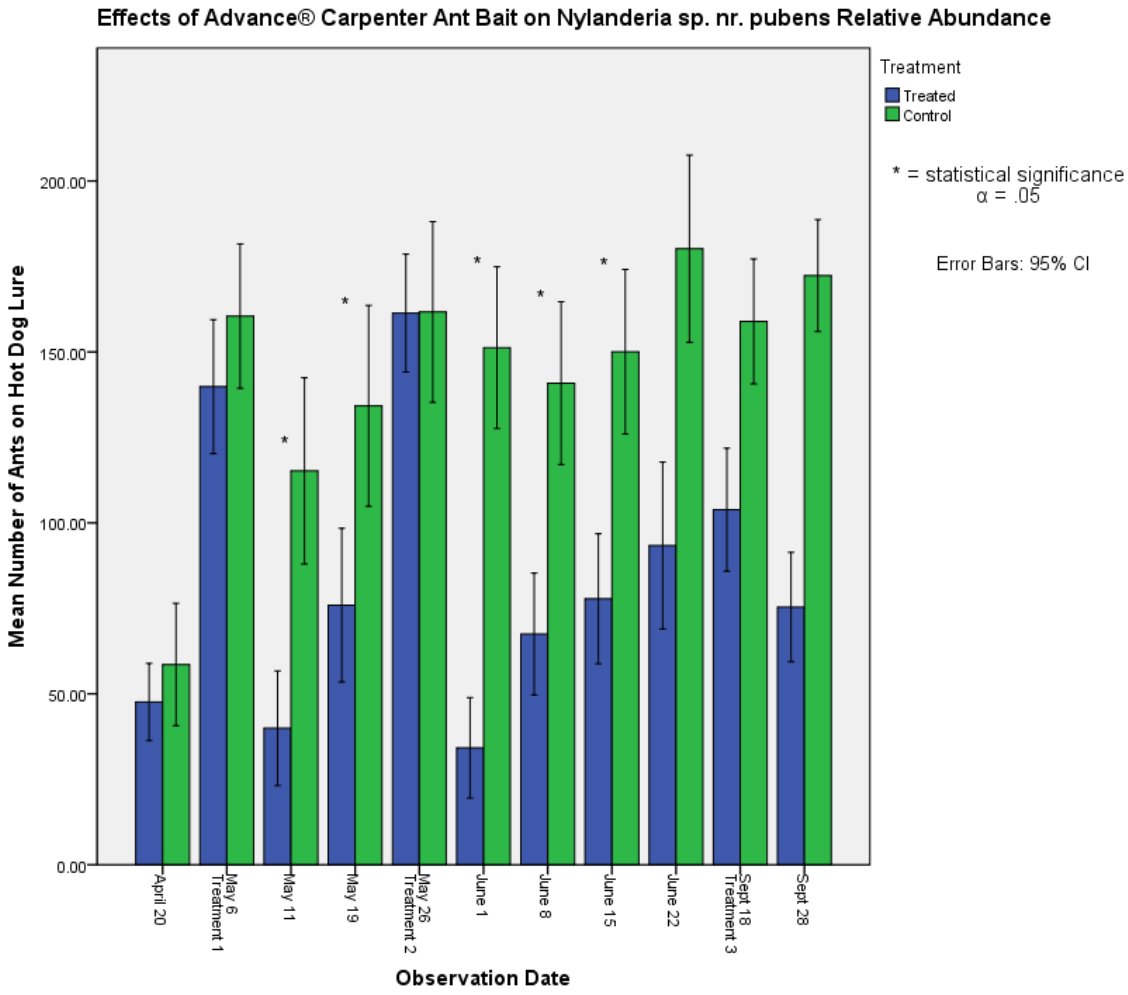
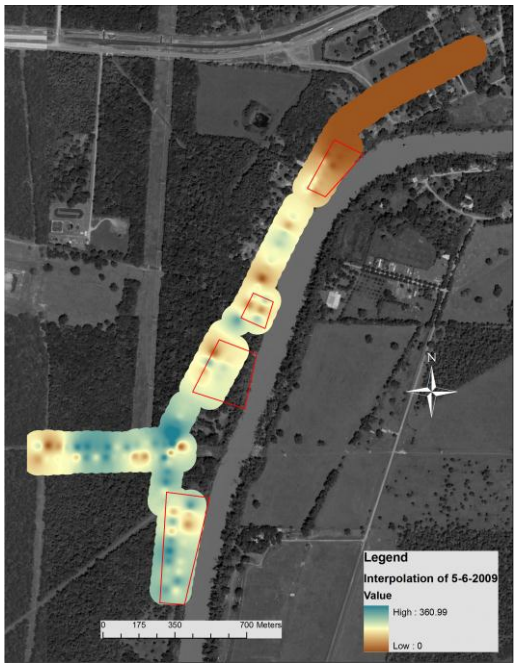
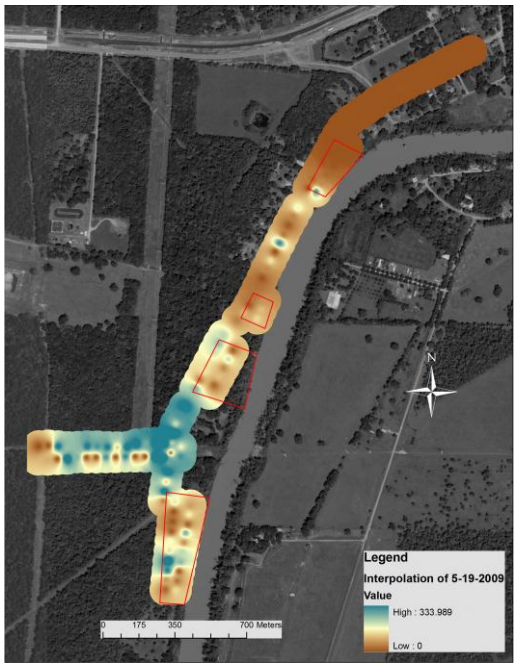


Fig. 11. Analysis of differences between treatments of Advance® Carpenter Ant Bait and control plots in East Columbia, Brazoria Co., Texas in 2009. Treatments were applied May 6th, May 26th, and Sept. 18th.

a) 

b) 

c) 

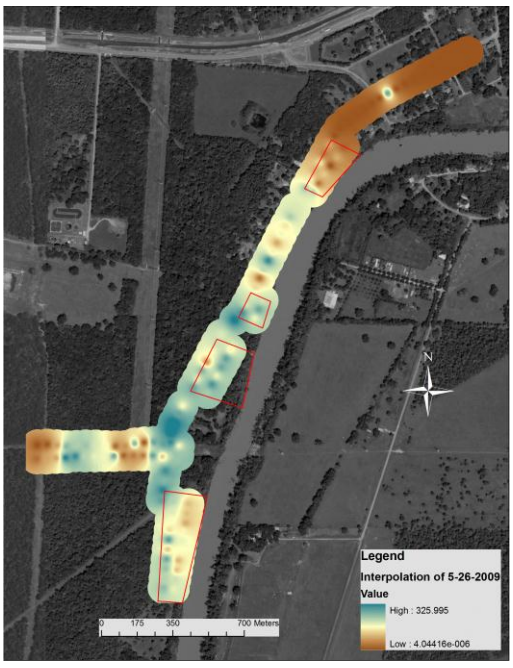
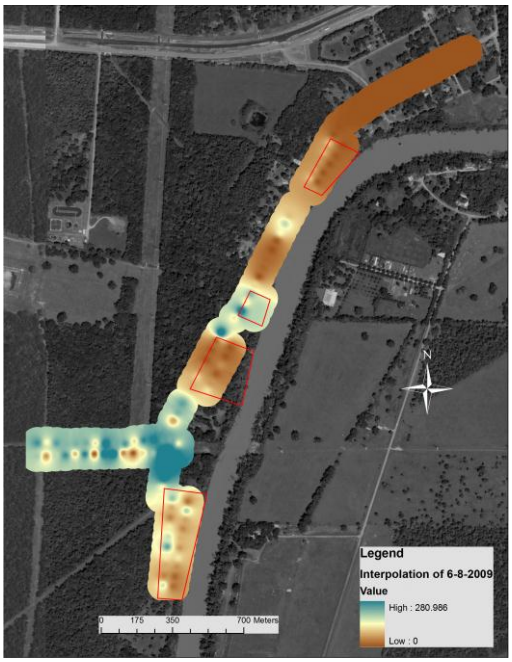
d) 

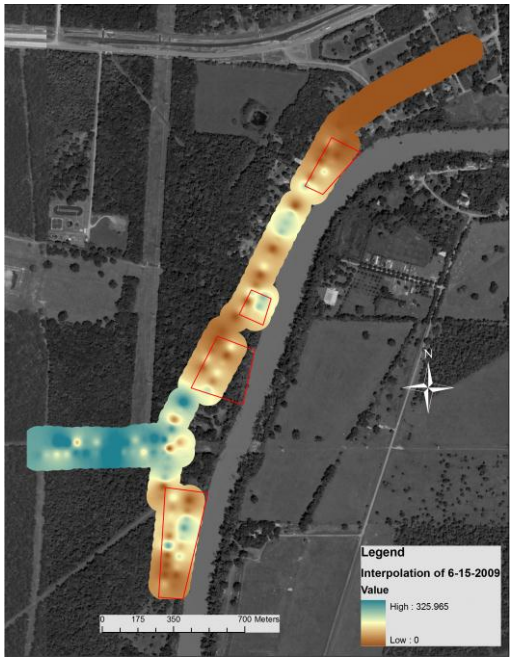
Fig. 12. GIS interpolation of *N. fulva* population density in treated and untreated plots in East Columbia, Brazoria Co., Texas in 2009. Blue coloration represents high densities of *N. fulva* and brown coloration represents low densities of *N. fulva*. Red boxes represent treated areas; a) May 6th treatment 1 applied, b) May 11th 58% control ($p < 0.001$), c) May 19th 37% control ($p < 0.001$), d) treatment 2 applied May 26th 0% control ($p = 0.984$).

Table 1. Effects of Advance® Carpenter Ant Bait on *N. fulva* relative abundance. Means with the same letter at each observation date are not significantly different according to Student's t Test (α=0.05).

Application	Observation Date	# Ants in Controls	# Ants in Treatments	% Control	p-value
	April 20th	60.12(a)	46.42(a)	-	0.725
Treatment 1	May 6th	160.26(a)	139.61(a)	-	0.311
	May 11th	112.83(a)	41.06(b)	58.23	<0.001
	May 19th	134.04(a)	73.00(b)	37.48	<0.001
Treatment 2	May 26th	161.69(a)	162.14(a)	-	0.984
	June 1st	151.25(a)	34.19(b)	77.46	<0.001
	June 8th	140.86(a)	67.49(b)	52.22	0.001
	June 15th	150.06(a)	74.47(b)	50.51	0.036
	June 22nd	180.19(a)	93.39(a)	-	0.129
Treatment 3	Sept. 18th	158.92(a)	108.24(a)	-	0.996
	Sept. 28th	172.34(a)	75.37(a)	-	0.430

a) 

b) 

c) 

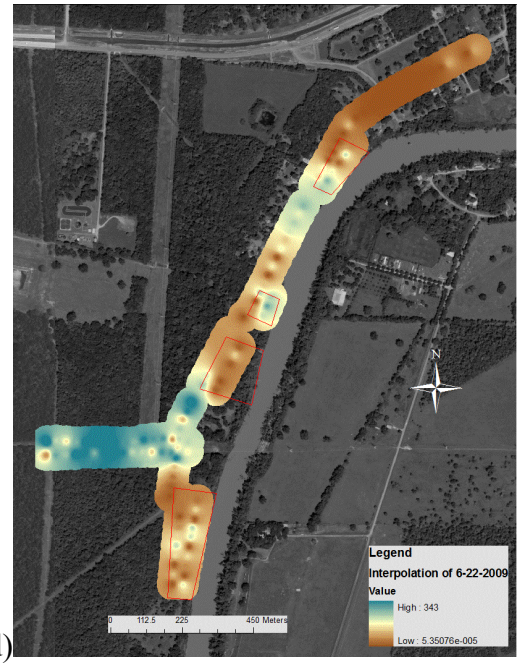
d) 

Fig. 13. GIS interpolation of *N. fulva* population density in treated and untreated plots in East Columbia, Brazoria Co., Texas in 2009. Blue coloration represents high densities of *N. fulva* and brown coloration represents low densities of *N. fulva*. Red boxes represent treated areas. a) June 1st 77% control ($p < 0.001$), b) June 8th 52% control ($p < 0.001$), c) June 15th 51% control ($p = 0.036$), d) June 22nd 0% control ($p = 0.129$).

no significant difference between treatment and control plots (p = 0.129) (Fig. 13d). A third treatment was administered on September 18th when there was no significant difference between control and treatment plots (p = 0.996) (Fig. 14a). Treatment 3 yielded no statistical separation between treatment and control plots, therefore, monitoring was discontinued at that time.

a) 

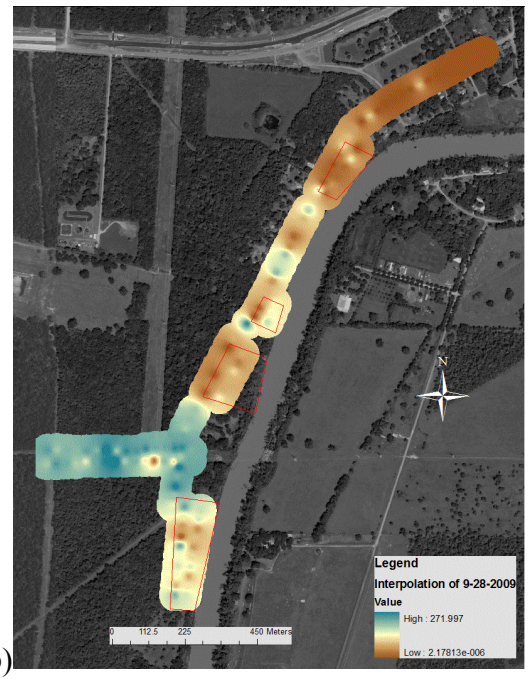
b) 

Fig. 14. GIS interpolation of *N. fulva* population density in treated and untreated plots in East Columbia, Brazoria Co., Texas in 2009. Blue coloration represents high densities of *N. fulva* and brown coloration represents low densities of *N. fulva*. Red boxes represent treated areas. a) Sept. 18th 0% control (p = .996), b) Sept. 28th 0% control (p=0.430).

Conclusions

Nylanderia fulva is an extreme nuisance pest that is expanding its range across Texas at a rapid pace (24 counties in 10 years). These invasive ants are displacing ecologically

important organisms, disrupting the daily lives of residents and their companion animals, invading homes, businesses, and hospitals, and no single pesticide or cultural practice has proved to be adequate for their control. *Nylanderia fulva* displace native and non-native ant fauna probably through exploitative and interference competition. As demonstrated with *S. invicta* by Calixto et al. (2007), the successful population suppression of *N. fulva* would undoubtedly result in the resurgence of native ant populations. Further research is needed in the area of basic biology, behavior, and integrated pest management in order for a satisfactory solution to be determined. However, Simberloff (2003a) suggested that the need for intensive population biological research of newly invasive species is lower in priority relative to the need for early detection and expedient chemical and mechanical control.

Results reported herein revealed that ACAB treatments did not provide adequate control of *N. fulva* populations. Although 58% and 77% control was achieved 1 week post treatment 1 and 2 respectively (Table 1), the level of reduction of the population was unobservable without relative abundance measurement methods used. Remaining ants were still very abundant and levels of control were unacceptable to residents asked to assess results of treatment efforts. After only 3-4 weeks post treatment, the *N. fulva* population rebounded to pretreatment levels. These results were similar to Hooper-Bui and Rust (2000) findings regarding the efficacy of abamectin against *L. humile* in Riverside, California.

Utilization of GIS tools provided a satisfactory visual aid for presenting results of this research. These methods could serve as a practical application for consultants, pest

control operators, extension agents, and researchers. *N. fulva* populations in untreated controls were shown to be higher than neighboring treatment plots (Fig. 12), and this may explain the rapid re-infestation by *N. fulva*. Colonies, or portions of colonies, seem to be moving into treated properties once the original population is reduced because ACAB leaves no residue or residual control soon following application. Reinfestation probably occurred by budding from established colonies along field perimeters, similar to the study of *Pheidole megacephala* control using Amdro® by Reimer and Berdsley (1990). An alternative explanation is that, because this species has so many queens per colony, perhaps ACAB was not affecting the queens and they were able to produce enough offspring within the three weeks to compensate for colony losses.

Although a typical pesticide treatment cycle is only applied quarterly throughout the year, the rate of reinfestation or population rebound was so great with *N. fulva* that monthly applications of ACAB would be necessary. To maintain consistent, acceptable population levels it seemed that an overly frequent application of ACAB as a stand-alone treatment would be required.

It is important to note that if ACAB had been applied when the population densities were considerably lower (April 20th; Table 1), a higher level of control may have been achieved. In addition, 77% control, if maintained, may be adequate for other ants to reestablish and prolong *N. fulva* population suppression. However, in this trial no resurgence of other ant species was documented. Furthermore, this study only evaluated ACAB as a stand-alone treatment as a potential spot eradication tool potentially suitable for multiple land use sites. If ACAB had been used in conjunction with contact

insecticides (e.g. Termidor®SC, Phantom®SC, and TopChoice®) a successful management program may have been achieved. This experiment could have been strengthened by documenting use in repeated treatments over several locations with periodic plot monitoring over a longer period of time (2-3 years). However, results are consistent through treatments and more efficacious management strategies using broadcast-applied ant bait formulations needs to be explored immediately.

Insect growth regulators (IGRs) prevent the replacement of workers through disruption of molting of immature colony members, a reduction in egg production, and a brood shift to sexual forms (Banks 1990). Fenoxycarb, pyriproxyfen, diflubenzuron, and methoprene are candidate IGRs for the control of *N. fulva*. A successful decrease in workers and brood of *N. fulva*, and the subsequent resurgence of native ant fauna was observed in a field trial using diflubenzuron, a chitin synthesis inhibitor in Valle, Colombia (Chacon de Ulloa et al. 2000). Laboratory experiments provided evidence that treatment of *N. fulva* with fenoxycarb and methoprene, juvenile hormone analogues, resulted in a significant reduction in worker ant abundance and a decrease in egg production (Chacon de Ulloa et al. 1994). Although IGRs act slowly to eliminate colonies, 4 to ≥8 weeks (Banks 1990), it may be the best way to achieve population level management for *N. fulva*. Furthermore, Banks (1990) showed that corncob grit (ground corncobs) retains only 15% of IGRs, and suggests that puffed cereal-type pellets such as defatted corn grit (oils separated from ground corncobs), rice, or wheat are more appropriate with these toxicants. Based on empirical evidence, it does not appear that *N. fulva* is attracted to oil based baits (Drees et al. 2010a), and to date, has shown no

response to IGR treatment attempts (pers. com. Drees and Paul Nester). Given that the proprietary bait matrix of ACAB is attractive to *N. fulva*, it should be paired with an IGR or other more effective active ingredient and tested in the field for effectiveness.

To conserve native biodiversity, spot eradication of *N. fulva* is crucial. Causton et al. (2005) demonstrated a successful spot eradication program for *Wasmannia auropunctata* from ~21 ha on Marchena Island in the Galapagos archipelago using Amdro® (hydramethylnon). *Solenopsis geminata* and *Pheidole megacephala* Forel have also been eradicated from small (3 ha and 10 ha respectively) infestations in Australia (Hoffmann and O'Connor 2004). Following the eradication of *P. megacephala*, native ant abundance and species richness rebounded to identical levels as uninfested areas (Hoffmann 2010). Therefore, development of effective management techniques for *N. fulva* is immediately necessary for conservation efforts. An area wide treatment of entire populations should provide the greatest chance of spot eradication (Sheffrahn and Warner 2011).

Future GIS analysis of data could depict the interspecific competition between *N. fulva* and other ant species, particularly RIFA. There were only three ant species that have been found coexisting with *N. fulva* in this study, *Pheidole* sp., *Solenopsis* (*Diplorhoptrum*) *molesta*, and *Brachymrmex* sp.; all others (*Crematogaster* sp., *Dorymyrmex* sp., *Forelius* sp., *Monomorium minimum*, and *S. invicta*) were displaced and found only outside of the *N. fulva* infestation. Furthermore, a reliable indicator of the absence of *N. fulva* in Texas landscapes is the presence of *S. invicta.*

CHAPTER IV

DIEL AND SEASONAL FORAGING PREFERENCE OF *Nylanderia fulva* (HYMENOPTERA: FORMICIDAE) IN TEXAS

Introduction

Nylanderia fulva, is a new invasive pest ant species in Texas. *Nylanderia fulva* is a tramp ant species, polygynous, unicolonial, and appears to reproduce by colony budding (Hölldobler and Wilson 1990, Passera 1994). In areas infested by *N. fulva*, large numbers of ants accumulate in electrical equipment, causing short circuits resulting in equipment failure (Meyers 2008). *Nylanderia fulva* is an ecologically dominating species displacing native and non-native ant species, attacking commercial apiaries, infesting structures such as homes, businesses, and hospitals, and utilizing human commerce to rapidly increase their distribution. Diet preference studies for *N. fulva* are limited to a laboratory study by Cook et al. (2010) and field observations by Zenner de Polania (1982) and Zenner de Polania and Bolanos (1985). Confirming diet preferences of Texas populations of *N. fulva* in the field allows for the development of efficient collection and monitoring techniques and contributing to our understanding of the best ingredients for bait matrices suitable for attracting *N. fulva*. It is also undetermined when daily peak foraging activity occurs for *N. fulva*. This project provides data related to seasonal foraging preference, diel activity, and temperature thresholds for *N. fulva* foraging in Texas.

In social insects, colony-level comparison of resources arises without the direct comparison by all individuals responding to food or nest quality (Robinson et al 2009). This is achieved by individuals deciding whether to commit to a resource or to seek out better options. It is believed that individual foragers have the capacity to store information and use it to make foraging decisions (Traniello 1989). Therefore, ants are capable of making colony level choices when selecting resources and individual choices of workers reflect the nutrient need of the colony (Robinson et al. 2009).

Based on information regarding the nutrient preference of *N. fulva* it is known that, in a laboratory setting, *N. fulva* prefer a carbohydrate-rich diet with a protein to carbohydrate ratio (p:c) of ~1:2 (Cook et al. 2011). This is consistent with a field study of *Nylanderia melanderi* which determined the macronutrient preference of carbohydrates (diluted syrup) over protein food sources (tuna) (Lynch et al. 1980). However, data suggests that preferences for various nutrients essential for metabolic processes may shift seasonally in the field for *N. fulva* (Cook et al. 2011).

Ants tend to have a distinct daily foraging schedule, either diurnal or nocturnal (Hölldobler and Wilson 1990), such as the changeover of ant assemblages at dusk in Australia as a way of partitioning resources (Wilson 1971). These daily cycles are based on circadian rhythms but can be overridden based on colony hunger or environmental changes as evident in *Formica* sp. and *Paraponera clavata* (McCluskey and Soong 1979, Rosengren and Fortelius 1986, McCluskey and Brown 1972, Harrison and Breed 1987). *Nylanderia melanderi* is known to be mostly diurnal in Maryland, with a niche breadth (actively foraging) from April to early December (Lynch et al. 1980), whereas *Solenopsis xyloni*

is a nocturnal forager (Hooper and Rust 1997). Determining the foraging schedule of a species can have implications related to proper management and collection techniques.

The diet of *Solenopsis invicta*, the red imported fire ant (RIFA), changes seasonally (Tschinkel 2006). In the laboratory, most ant species can subsist on a diet of sugar water and an insect protein source (Tschinkel 2006). Cook et al. (2010) determined that RIFA were most attracted to foods with equal to moderately protein-biased diets. In the field, RIFA are attracted by a wide array of foods (Tschinkel 2006), but demonstrate a seasonal shift in nutrient preference, recruiting to carbohydrates in lower temperatures (mean =17°C), and proteins in the higher temperatures (mean = 25°C) (Stein et al. 1990). This is likely due to the presence of brood (eggs, larvae, and puape) during warmer seasons. Carbohydrates are consumed by workers and proteins are fed to larvae (Abbott 1978, Cannon and Fell 2002, Cook et al. 2010, Dussutour and Simpson 2009, Hölldobler and Wilson 1990, Stradling 1978, Tschinkel 2006). Through worker communication, ants are able to make colony level decisions about food requirements and adjust their harvesting strategy according to nutrient demands among individuals within the colony (Dussutour and Simpson 2009). For example, *Linepithema humile* focus their attention on proteins during queen oviposition periods and during larval development, and on carbohydrate foods when males and workers are hatching (Abril et al. 2007). Ultimately, colony level diet selection allows for production of brood, specifically the next generation of reproductives (Hölldobler and Wilson 1990). Therefore, it is not surprising that a larger ratio of larvae to workers results in an emphasis placed by foragers on collecting protenaceous foods (Hölldobler and Wilson 1990).

Nutritional preferences might also reflect the relative availabilities of particular nutrients in the environment (Stein et al. 1990, Hahn & Wheeler 2002, Kay 2004). Worker ants are attracted more strongly to concentrated rather than to dilute solutions (Dussutor and Simpson 2008). Furthermore, recruitment rates depend on food type, concentration, and hunger level (Dussutor and Simpson 2008).

Ant foraging activity can be constrained by temperature and water stress (Traniello 1989, Tschinkel 2006). Temperature thresholds for foraging activities reflect the season of relative food abundance for that species (Bernstein 1979). *Nylanderia melanderi* is known to have a lower foraging threshold of 15°C with a peak between 25 and 30°C (Lynch et al. 1980). *Linepithema humile* workers are active between 5 and 35°C with the greatest intensity between 15 and 30°C (Markin 1970). Porter and Tschinkel (1987) reported that RIFA foraging is essentially non-existent below 15°C and above 43°C with a maximal rate of foraging between 22 and 36°C. It was also determined that the best predictor for foraging rates of RIFA is soil temperature at a depth of 2 cm, and that relative humidity, saturation deficits, soil moisture, and wind were unrelated to RIFA foraging rates (Porter and Tschinkel 1987). However, the higher the relative humidity, the greater the temperature tolerance of ant species such as *Pheidole militicida*, *Formica polyctena* and *Prenolepis impairs* (Hölldobler and Wilson 1990, Schumacher and Whitford 1974, Hölldobler and Moglich 1980, Rosengren 1977, Talbot 1943, 1946, Briese and MaCauley 1980). Every species operates within critical upper and lower temperature-humidity thresholds, but foragers are more acutely affected by temperature and humidity than colony members within the microenvironment of the nest (Hölldobler and Wilson 1990). Temperature thresholds inhibiting foraging may not have substantial deleterious effects on the colony unless they

persist to the point where nutrient stores are exhausted. Added variance comes from microhabitat specialization and competition avoidance (Hölldobler and Wilson 1990) and rainfall typically halts most ant foraging (Hölldobler and Wilson 1990, Hodgson 1955, Lewis et al. 1974, Skinner 1980).

Ant species ecologically similar to *N. fulva* have a largely liquid diet, but some will take solid arthropod prey. *Nylanderia* spp. foragers commonly tend phloem-fluid feeding hemipterous insects such as aphids, scale insects, whiteflies, planthoppers (Humphreys 1998), and mealybugs (Carver et al. 1987) for their honeydew. *Nylanderia fulva* have been observed feeding on sweet parts of plants including nectaries, extra-floral nectaries, and damaged and over-ripe fruit. The diet of *N. fulva* consists of liquid carbohydrates (honeydew secreted by hemipterans) and solid animal protein (mostly by predation on insects, spiders, and even non-arthropod animals) (Zenner de Polania 1982, Zenner de Polania and Bolanos 1985). In *Linepithema humile* and *Paratrechina longicornis* (behaviorally similar species to *N. fulva*) ≥99% of food brought to the nest by foragers is honeydew and nectar (Markin 1970, Wetterer et al. 1999). The solid diet of *L. humile* is composed of mostly aphids and thrips (Abril et al. 2007, Markin 1970). *Anoplolepsis longipes* prefer liquids over solid foods (Rao and Veeresh 1991a), and <1% of returning *Campanotus pennsylvanicus* foragers carry solid matter in their mandibles (Cannon and Fell 2002). Tennant and Porter (1991) reported that 80% of RIFA and *Solenopsis geminata* foraging trips yielded liquid returns regardless of season. These fluids contained glucose, fructose, and amino acids. They concluded that the most likely sources of these fluids were plant sap and hemipteran honeydew.

High tempo ants (those who exhibit relatively high foraging rates) tend to prefer strongly carbohydrate biased diets, but the 1:2 ratio of proteins to carbohydrates preferred by *N. fulva* in laboratory experiments is more similar to low tempo species (Cook et al. 2011, Davidson 1997, Oster and Wilson 1978). Investment in carbohydrate-rich resources contributes to competitive performance through an increase in aggression, common in invasive species (Grover et al. 2007). Wilder et al. (2011) suggested that the food-for-protection mutualism between *S. invicta* and honeydew-producing Hemiptera increased their competitive performance, and ultimately played a fundamental role in the species successful invasions.

It is important to simultaneously investigate basic behavioral studies and applied management techniques when researching pestiferous invasive species in order to mitigate further distribution. The need for intensive research regarding population dynamics of newly invasive species is lower in priority relative to the need for early detection and expedient chemical and mechanical control (Simberloff 2003a). Knowledge of a species' diet can be used to more effectively monitor their spread or detection in a new habitat as well as development of species specific bait matrices, and should be a high priority when investigating invasive species.

Materials and Methods

A field study coupling diel foraging activity and diet and lure preference observations was conducted in East Columbia, Texas throughout the four seasons (winter, fall, spring, and summer). The aim of this study was to gain insight into the

nutritional preferences, temperature tolerances, and the optimal time to sample for *N. fulva*. Voucher specimens from this study were deposited in the Center for Urban and Structural Entomology and Texas A&M University Insect Collection (Voucher #688, Department of Entomology, Texas A&M University, College Station, Texas).

In a choice experiment, *N. fulva* were presented with multiple food lures or artificial diets which were complimentary in nutrient profiles and then allowed to select their preferred intake of nutrients (Table 2 and Table 3). *Nylanderia fulva* were offered 8 food lures replicated 12 times through the four seasons. Sampling dates were chosen based on the mid date (± 7 d) for each season. Six replicates were placed in the shade to minimize lure avoidance because of high temperatures. Six more replicates were placed in direct sunlight to determine temperature thresholds for foraging activity. A 4 mm slice of hot dog (Bar-S) and 1/8 teaspoon of each of the other lures were placed in randomly selected wells in a 10 well watercolor mixing dish (PleinAirCo Artist Materials). Blank wells in the mixing dishes served as controls to account for random foraging. Replicates were checked a minimum of 10 times for a 24 hour period, and a picture was taken of each mixing dish for counting ants in each well. Time of daily foraging periods has been compared to soil surface temperature for at least 44 different ant species in 21 different publications (Bernstein 1979, Hölldobler and Wilson 1990). Therefore, ground temperature was measured using a Fisher Scientific Traceable® Light Meter Pen (Cat No. 15-078-189) during each observation to determine whether *N. fulva* foraging activity is constrained by soil temperature.

Table 2. Components of experimental diets. Amounts are based on ~293.5 g macronutrient total weight. The whole egg powder contributed a constant amount of 62.4 g of lipids (fats and sterols) to each diet.

Diet	Calcium Caseinate (g)	Whey Protein (g)	Egg Powder (g)	Sucrose (g)	Color
p19:c57	15.6	17.2	62.4	196.4	Red
p33:c43	38.8	41.6	62.4	148.0	Yellow
p42:c32	57.2	62.0	62.4	111.2	Green
p54:c18	81.2	89.6	62.4	65.6	Blue

Table 3. Carbohydrate, protein, and fat content of food lures. Percentages are based on manufacturer's labels, and extraction assays for scrambled chicken egg and mealworms.

Food Lure	Protein:Carbohydrate:Fat
Mint Apple Jelly	0:65:0
Hot Dog	10:7:26
10% Honey Water	0:8:0
Peanut Butter	25:22:50
Mealworm	20:7:12
Scrambled Chicken Egg	35:2:63
Vegetable Oil	0:0:100
Water	0:0
Blank 1	0:0
Blank 2	0:0

Nylanderia fulva were offered 4 artificial diets consisting of known ratios of proteins to carbohydrates (P:C) (Table 2). Consistent with the food lure trial, 6 replicates were placed in the shade, and 6 replicates were place in direct sunlight. The dietary protein component was an approximate 1:1 mixture of whey protein concentrate (Jarrow Formulas®) and calcium caseinate (True Protein). Whole egg powder (Honeyville® Farms) provided a source of essential lipids, including sterols (Table 2). The sole source of digestible carbohydrate (henceforth only carbohydrate) in our experimental diets was sucrose (Great Value™ Pure Cane Sugar). Each diet was dyed a

separate color (Adams Extract) for ease of differentiation (p19:c57 = red, p33:c43 = yellow, p42:c32 = green, and p54:c18 = blue). Diets were dried using a Fisher Scientific Isotemp Oven set at 50°C for 12 hrs. Each diet was ground to 1 mm diameter granules through a no. 18 U.S.A. Standard Sieve. Each of the diets ($1/8^{th}$ of a teaspoon) was placed in randomly selected wells in a watercolor mixing dish. Observations were conducted as described above.

Prior to each analysis, data were checked for normality and equal variances. Using relative abundance of *N. fulva* foraging workers as an indication of preference, an analysis of variance (ANOVA) (SPSS 19) was used to measure differences between lures (α=0.05). Post hoc differences among means were established using Tukey's HSD. Parametric statistics were used to conduct all analyses. Foraging thresholds were measured by comparing the number of ants on hot dogs (HD) with ground temperature. Peak foraging activity was determined by averaging the ground temperature for the 6 highest ant counts on HD (the traditional food lure used for *N. fulva* relative abundance).

Results

Food lure trials showed that *N. fulva* preference was variable across seasons and between cover-types (shade vs. sun) within a season. In the spring, *N. fulva* showed a preference for both mint apple jelly (MAJ) and HD when cover-type results were combined (Table 4, Fig. 15). However, *N. fulva* preferred HD in the sun and MAJ in the shade (Table 5 & 6, Fig. 16 & 17). Summer results showed an opposite lure preference than spring results in that *N. fulva* had a strong preference for HD in the shade. The

Table 4. Food lure preference of *N. fulva* across all seasons with cover type results combined. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Lure	Spring	Summer	Fall	Winter
Mint Apple Jelly	45.84 (a)	21.52 (b)	54.23 (a)	8.16 (a)
Hot Dog	40.85 (a)	48.62 (a)	22.39 (c)	3.62 (bc)
Honey Water	11.15 (bc)	3.02 (cde)	34.94 (b)	5.91 (ab)
Peanut Butter	15.86 (b)	8.56 (cd)	22.91 (c)	2.49 (cd)
Mealworm	7.59 (cd)	9.19 (c)	7.05 (de)	0.22 (d)
Scrambled Chicken Egg	5.55 (cde)	2.06 (de)	15.15 (cd)	0.28 (d)
Vegetable Oil	2.17 (de)	0.96 (e)	3.32 (e)	0.12 (d)
Water	0.86 (e)	0.73 (e)	2.09 (e)	0.10 (d)
Blank 1	1.01 (e)	0.60 (e)	1.29 (e)	0.05 (d)
Blank 2	0.83 (e)	0.42 (e)	1.28 (e)	0.03 (d)
df	9	9	9	9
Mean Square	51382.47	50025.27	50776.76	1024.30
F value	138.52	98.65	85.67	22.69
P value	<0.001	<0.001	<0.001	<0.001

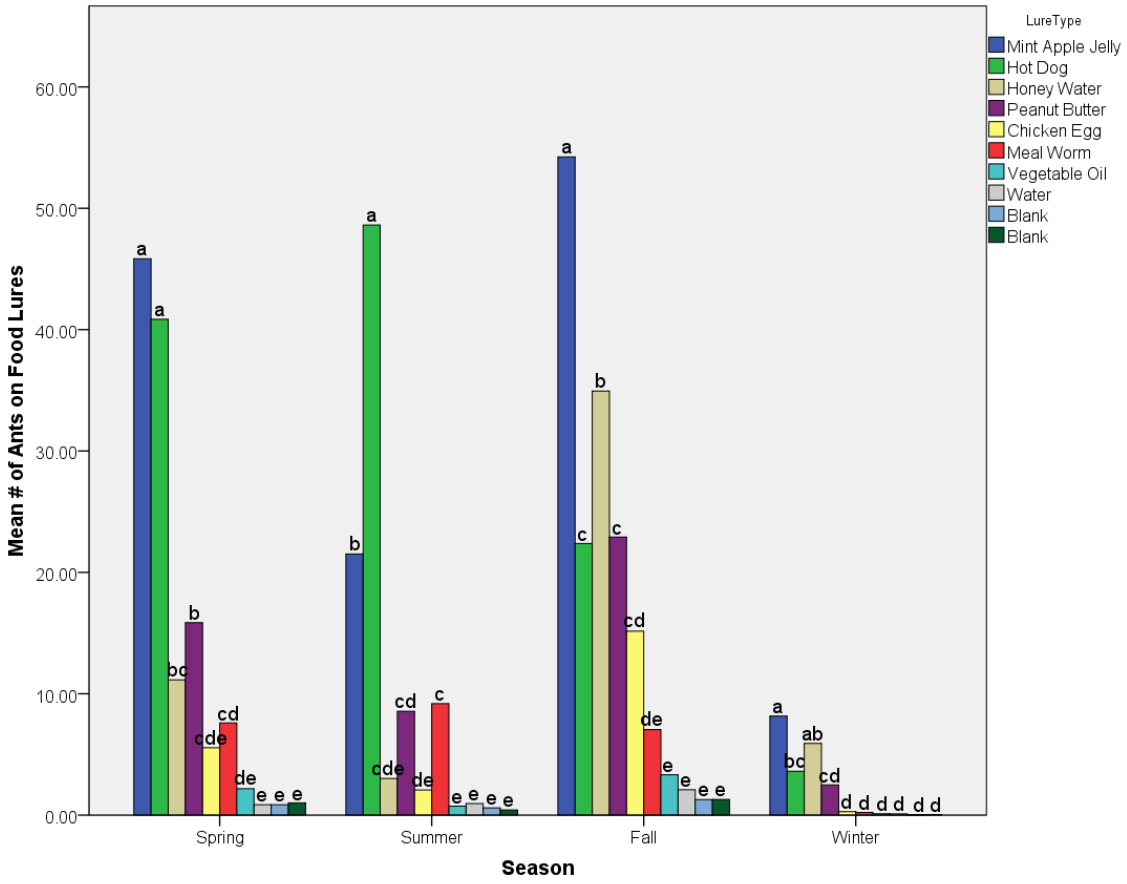


Fig. 15. Food lure preference of *N. fulva* across all seasons and cover types. *N. fulva* preference was variable between seasons and cover-types (sun and shade). Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Table 5. Food lure preference of *N. fulva* across all seasons in the sun. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Lure	Spring	Summer	Fall	Winter
Mint Apple Jelly	9.78 (b)	36.02 (a)	40.36 (a)	14.80 (a)
Hot Dog	53.41 (a)	31.29 (a)	33.05(ab)	7.15 (bc)
Honey Water	0.99 (d)	5.13 (bc)	31.64 (ab)	11.19 (ab)
Peanut Butter	7.43 (b)	12.50 (b)	30.49 (ab)	4.90 (cd)
Mealworm	7.85 (b)	2.73 (c)	9.47 (c)	0.42 (de)
Scrambled Chicken Egg	6.78 (bc)	1.07 (c)	22.32 (b)	0.47 (de)
Vegetable Oil	1.85 (cd)	0.36 (d)	5.51 (c)	0.22 (de)
Water	0.41 (d)	0.52 (d)	2.44 (c)	0.17 (de)
Blank 1	0.33 (d)	0.50 (d)	1.18 (c)	0.08 (de)
Blank 2	0.22 (d)	0.37 (d)	1.01 (c)	0.03 (e)
df	9	9	9	9
Mean Square	23483.18	19791.83	19452.41	1750.34
F value	195.59	39.16	36.19	25.01
P value	<0.001	<0.001	<0.001	<0.001

Table 6. Food lure preference of *N. fulva* across all seasons in the shade. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Lure	Spring	Summer	Fall	Winter
Mint Apple Jelly	80.02 (a)	7.02 (c)	68.12 (a)	1.52 (a)
Hot Dog	28.16 (b)	65.95 (a)	11.46 (cd)	0.08 (b)
Honey Water	20.98 (b)	0.92 (c)	37.81 (b)	0.78 (ab)
Peanut Butter	23.84 (b)	4.61 (c)	15.33 (c)	0.08 (b)
Mealworm	7.37 (c)	15.65 (b)	4.77 (cd)	0.02 (b)
Scrambled Chicken Egg	4.39 (c)	3.06 (c)	7.99 (cd)	0.10 (b)
Vegetable Oil	2.48 (c)	1.11 (c)	1.13 (d)	0.02(b)
Water	1.29 (c)	1.40 (c)	1.74 (d)	0.03 (b)
Blank 1	1.40 (c)	0.83 (c)	1.57 (d)	0.02 (b)
Blank 2	1.75 (c)	0.34 (c)	1.37 (d)	0.02 (b)
df	9	9	9	9
Mean Square	56661.68	43939.18	39143.99	14.86
F value	187.69	110.49	69.27	4.91
P value	<0.001	<0.001	<0.001	<0.001

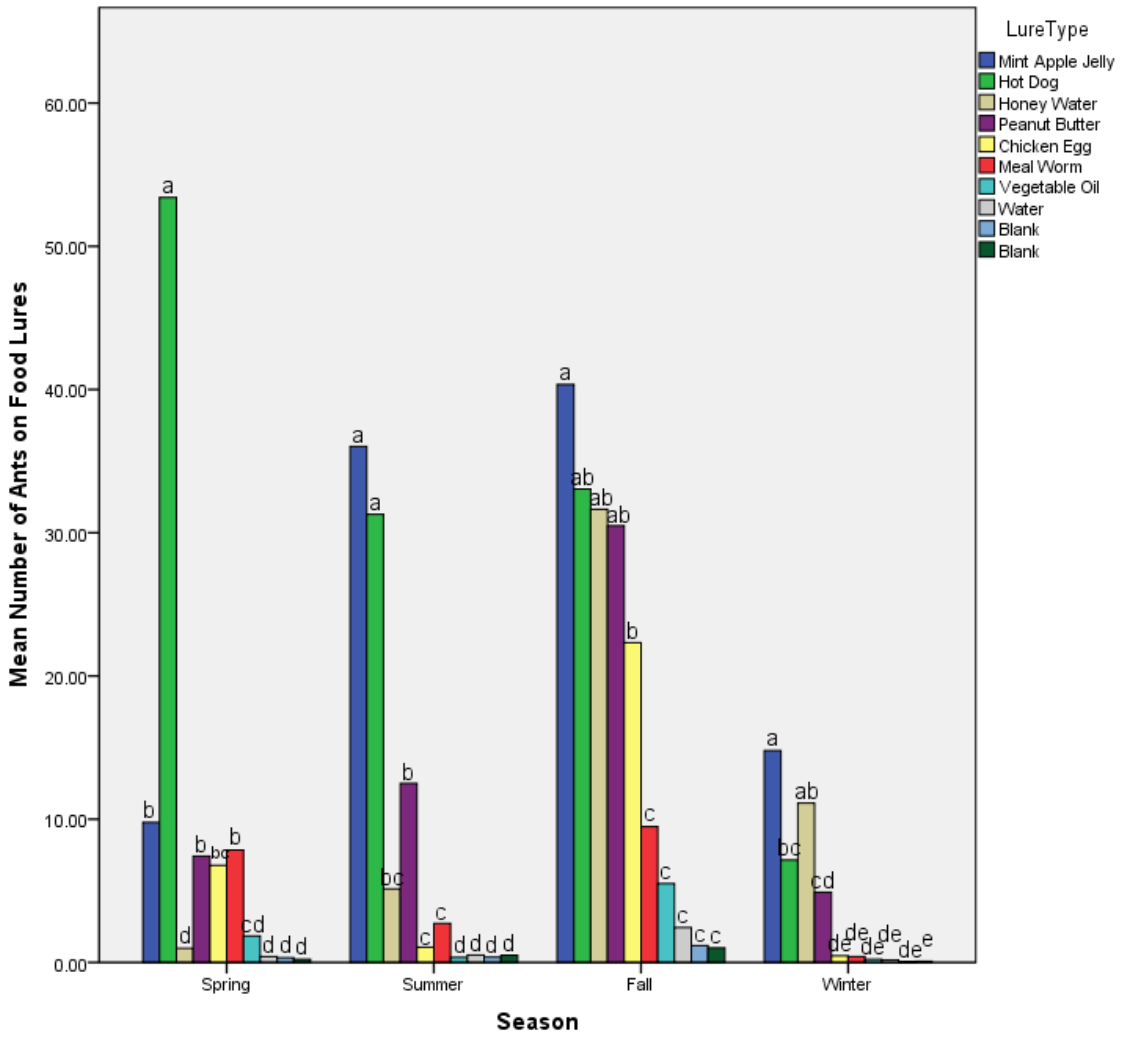


Fig. 16. Food lure preference of *N. fulva* across all seasons in the sun. *N. fulva* preference was variable between seasons and cover-types (sun and shade). Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

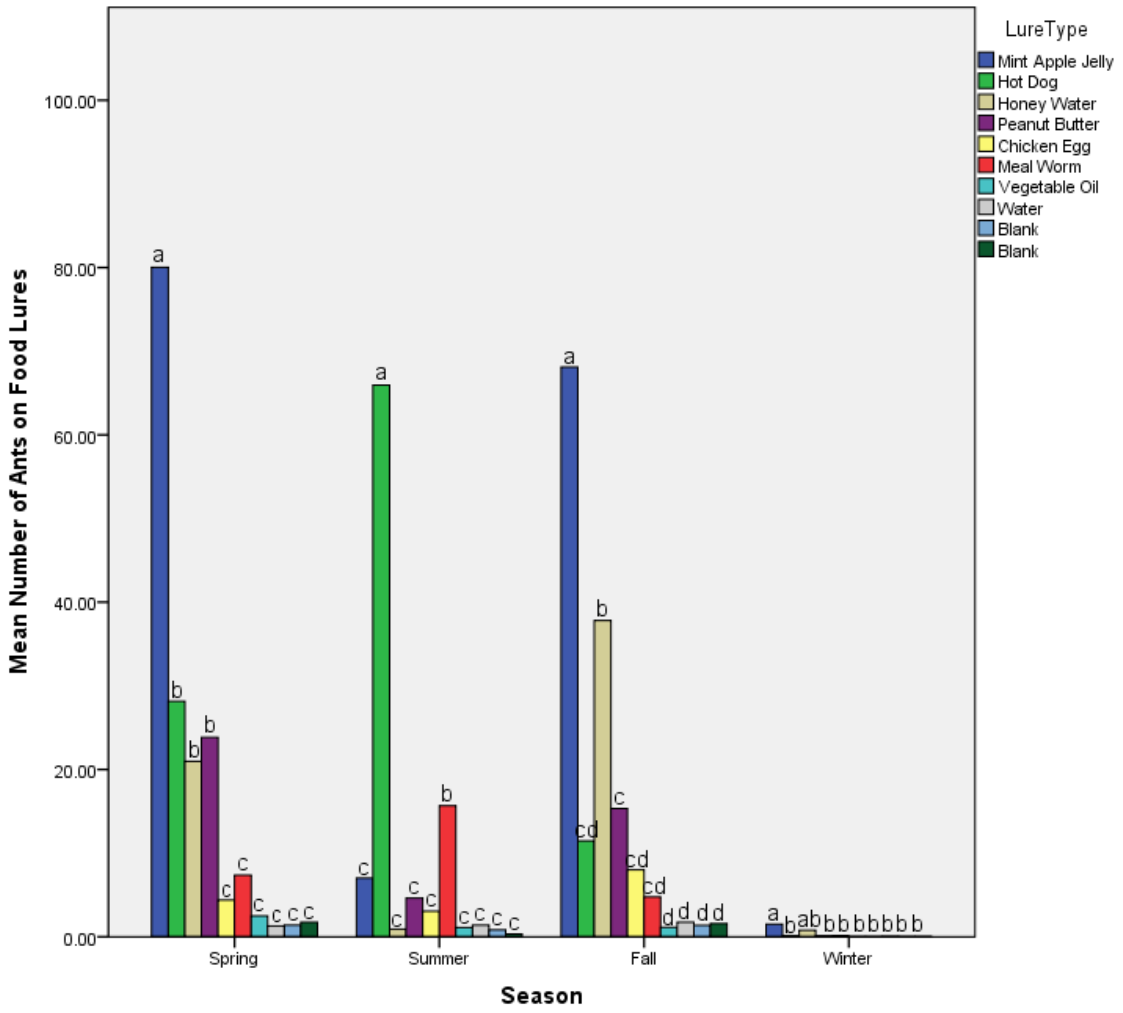


Fig. 17. Food lure preference of *N. fulva* across all seasons in the shade. *N. fulva* preference was variable between seasons and cover-types (sun and shade). Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

mean number of ants was highest for MAJ in the sun, but was not significantly different (α = 0.05) from HD. When cover-type results were combined, HD was the most preferred food lure. In the fall sampling, MAJ was preferred when cover-type results were combined. MAJ was also preferred in the sun but was not significantly different than HD, honey water (HW), or peanut butter (PB). In the shade MAJ attracted the most

foragers with HW being less attractant, but not significantly different. In the winter MAJ and HW were equally chosen in both cover-types.

Worker foraging intensity was significantly affected by dietary p:c ratio. Ants in this study preferred carbohydrate intake over protein. In spring, summer, and fall, *N. fulva* preferred the most carbohydrate rich diet (p19:c57) in both cover-types (Table 7, 8, & 9 Fig. 18, 19, & 20). However, in the winter sampling, *N. fulva* foraging was minimal and a preference was undetectable.

Figure 21 depicts foraging intensity on HD food lures as a function of ground temperature. Both sun and shade cover-type results illustrate the same trend. Peak foraging activity for *N. fulva* occurred at 28.24 ± 3.12°C. The lower foraging threshold was 9.95°C and the upper foraging threshold was observed at 37.26°C. *Nylanderia fulva* did not forage during the rain event, which occurred between 2 pm and 7 pm during the spring sampling (Fig. 22).

During the summer observations *N. fulva* foragers were crepuscular, foraging during the early morning and late afternoon hours (Fig. 22). During all other seasons *N. fulva* foraged closer to the middle of the day, when temperatures were between the measured thermal thresholds.

Table 7. Diet preference of *N. fulva* across all seasons with cover type results combined. ANOVA results for the winter sampling were not statistically significant and therefore, a post hoc analysis was not performed. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means in columns with the same letter within a season are not significantly different (α=0.05).

Diet	Spring	Summer	Fall	Winter
p:19c:57	25.49 (a)	2.81 (a)	2.60 (a)	0.49
p:33c:43	16.88 (b)	1.72 (b)	1.83 (b)	0.67
p:42c:32	17.76 (b)	1.14 (bc)	1.24 (bc)	0.50
p:54c:18	4.96 (c)	0.94 (c)	1.01 (c)	0.18
Blank	0.24 (c)	0.11 (d)	0.03 (d)	0.00
df	4	4	4	4
Mean Square	20204.38	116.69	142.89	8.84
F value	53.80	29.362	32.48	2.27
P value	<0.001	<0.001	<0.001	0.061

Table 8. Diet preference of *N. fulva* across all seasons in the sun. ANOVA results for the winter sampling were not statistically significant and therefore, a post hoc analysis was not performed. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Diet	Spring	Summer	Fall	Winter
p:19c:57	12.50 (a)	2.85 (a)	2.17 (a)	0.95
p:33c:43	6.99 (b)	1.50 (b)	1.33 (b)	1.33
p:42c:32	8.61 (ab)	1.28 (bc)	1.04 (b)	0.98
p:54c:18	2.26 (c)	0.57 (bc)	0.90 (b)	0.33
Blank	0.10 (c)	0.12 (c)	0.26 (c)	0.00
df	4	4	4	4
Mean Square	2370.40	49.26	46.33	17.50
F value	23.14	9.06	16.14	2.34
P value	< 0.001	< 0.001	< 0.001	0.055

Table 9. Diet preference of *N. fulva* across all seasons in the shade. ANOVA results for the winter sampling were not statistically significant and therefore, a post hoc analysis was not performed. ANOVA p-values are listed below each season. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

Diet	Spring	Summer	Fall	Winter
p:19c:57	38.48 (a)	2.78 (a)	3.04 (a)	0.03
p:33c:43	26.76 (b)	1.86 (b)	2.33 (ab)	0.00
p:42c:32	26.91 (b)	1.06 (c)	1.44 (bc)	0.02
p:54c:18	7.67 (c)	1.18 (bc)	1.12 (c)	0.02
Blank	0.39 (c)	0.11 (c)	0.03 (d)	0.00
df	4	4	4	4
Mean Square	23321.14	70.90	103.63	.012
F value	45.90	23.08	17.92	.88
P value	<0.001	<0.001	<0.001	0.475

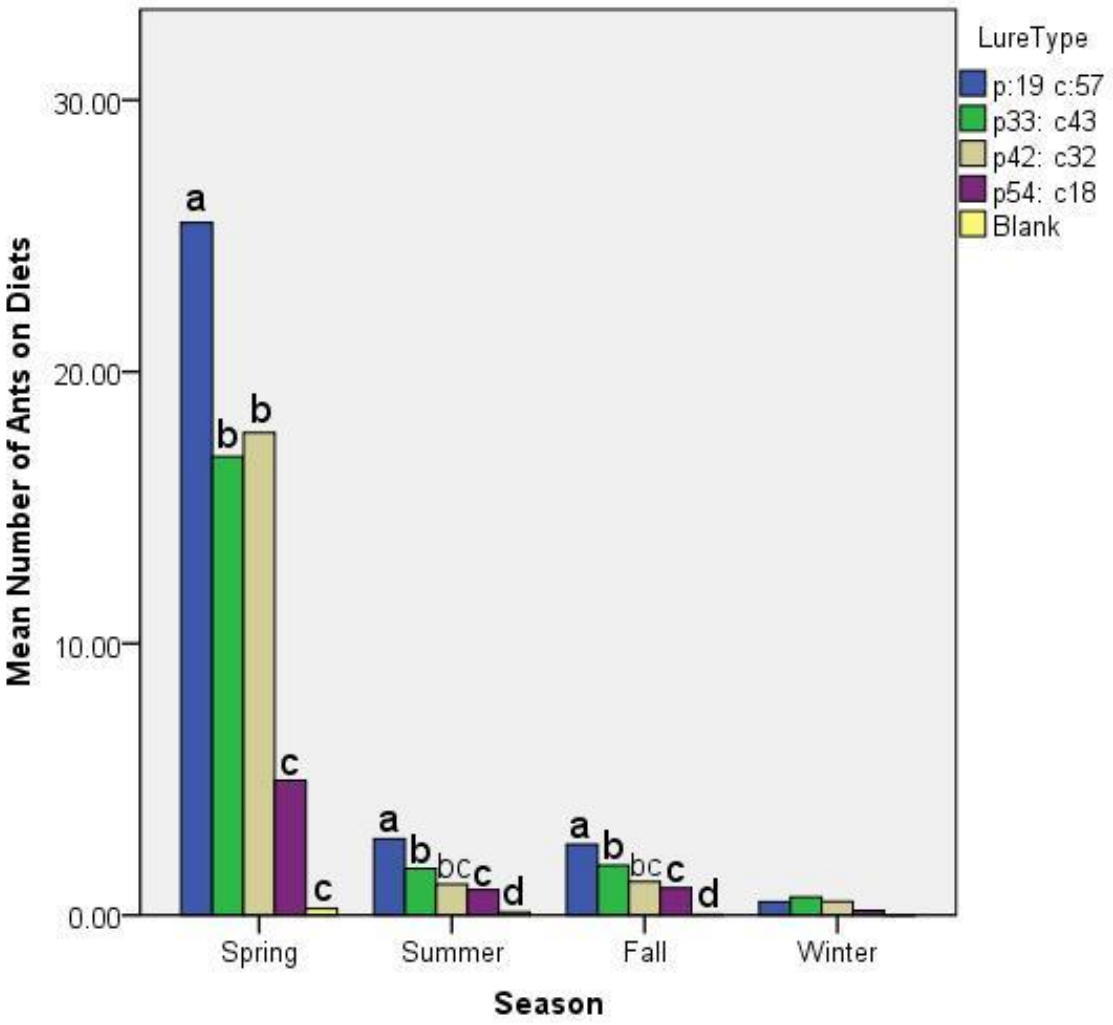


Fig. 18. Diet preference of *N. fulva* across all seasons and cover types. *N. fulva* preferred a carbohydrate rich diet through all seasons with the highest foraging activity in the spring. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

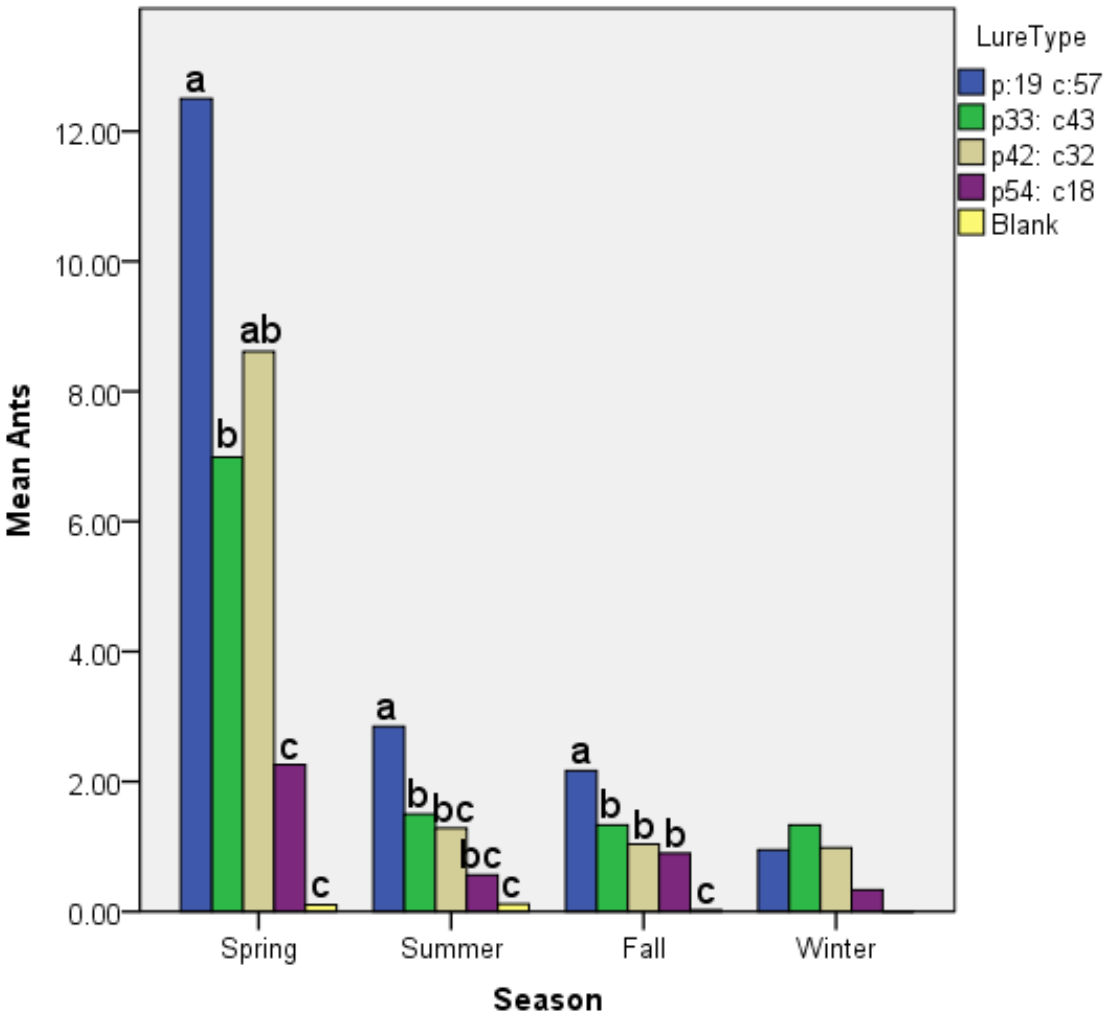


Fig. 19. Diet preference of *N. fulva* across all seasons in the sun. *N. fulva* preferred a carbohydrate rich diet through all seasons with the highest foraging activity in the spring. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

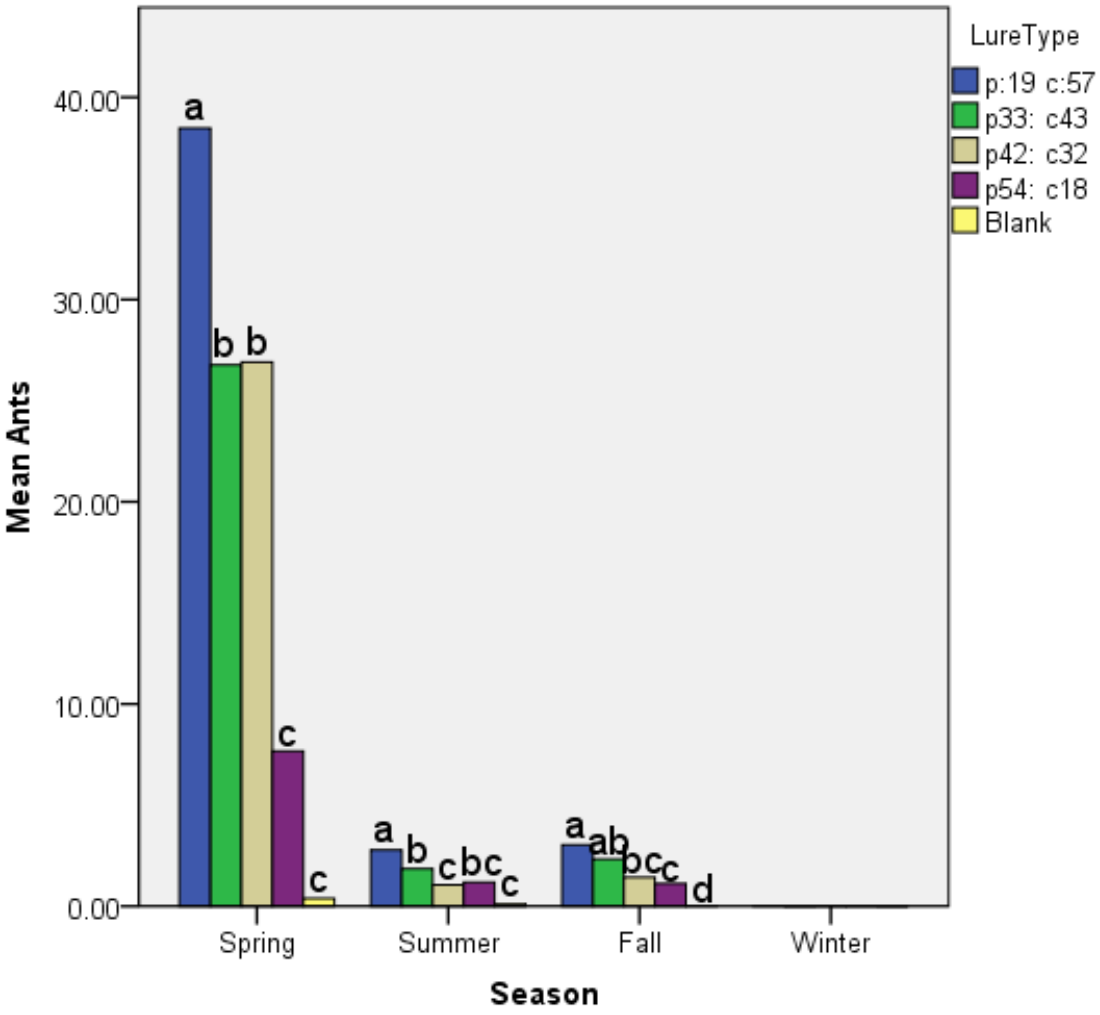


Fig. 20. Diet preference of *N. fulva* across all seasons in the shade. *N. fulva* preferred a carbohydrate rich diet through all seasons with the highest foraging activity in the spring. Mean separation was determined with Tukey's HSD. Means with the same letter within a season are not significantly different (α=0.05).

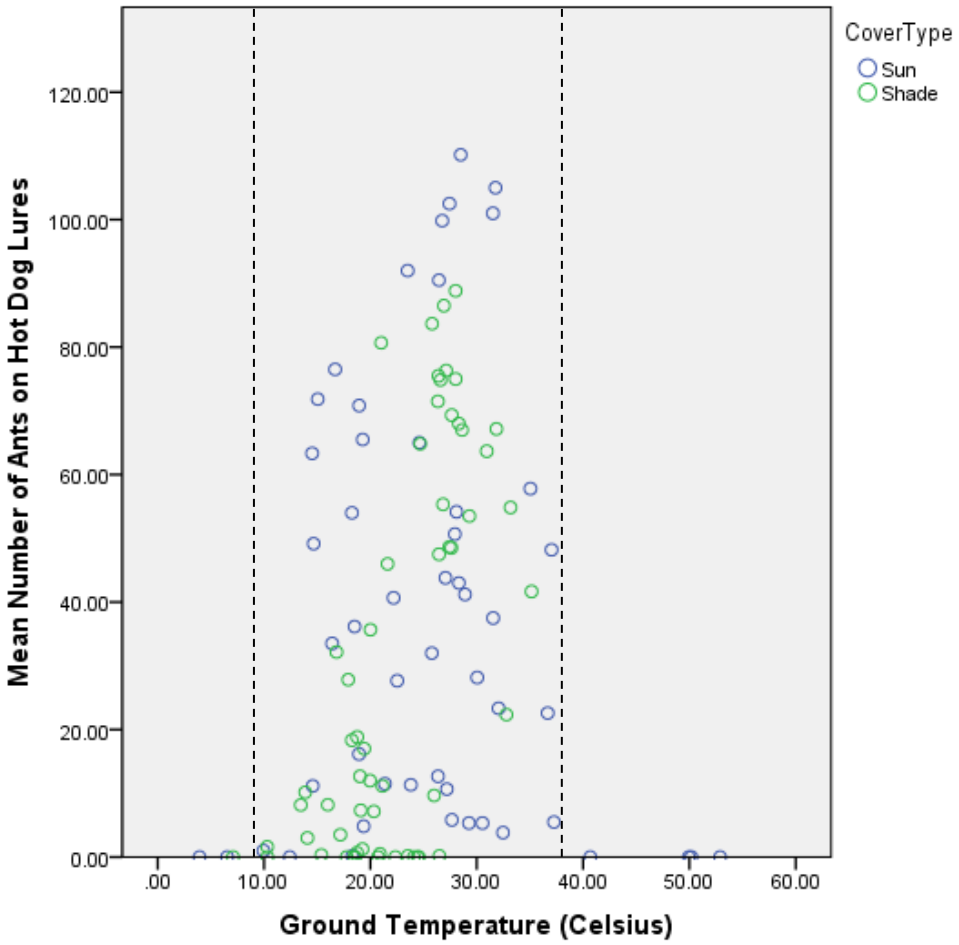


Fig. 21. Foraging temperatures of *N. fulva* on hot dog food lures. Dotted lines represent the foraging thresholds. Peak foraging activity occurred at 28.24 ± 3.12°C. The lower foraging threshold was found to be 9.95°C and the upper foraging threshold was observed at 37.26°C.

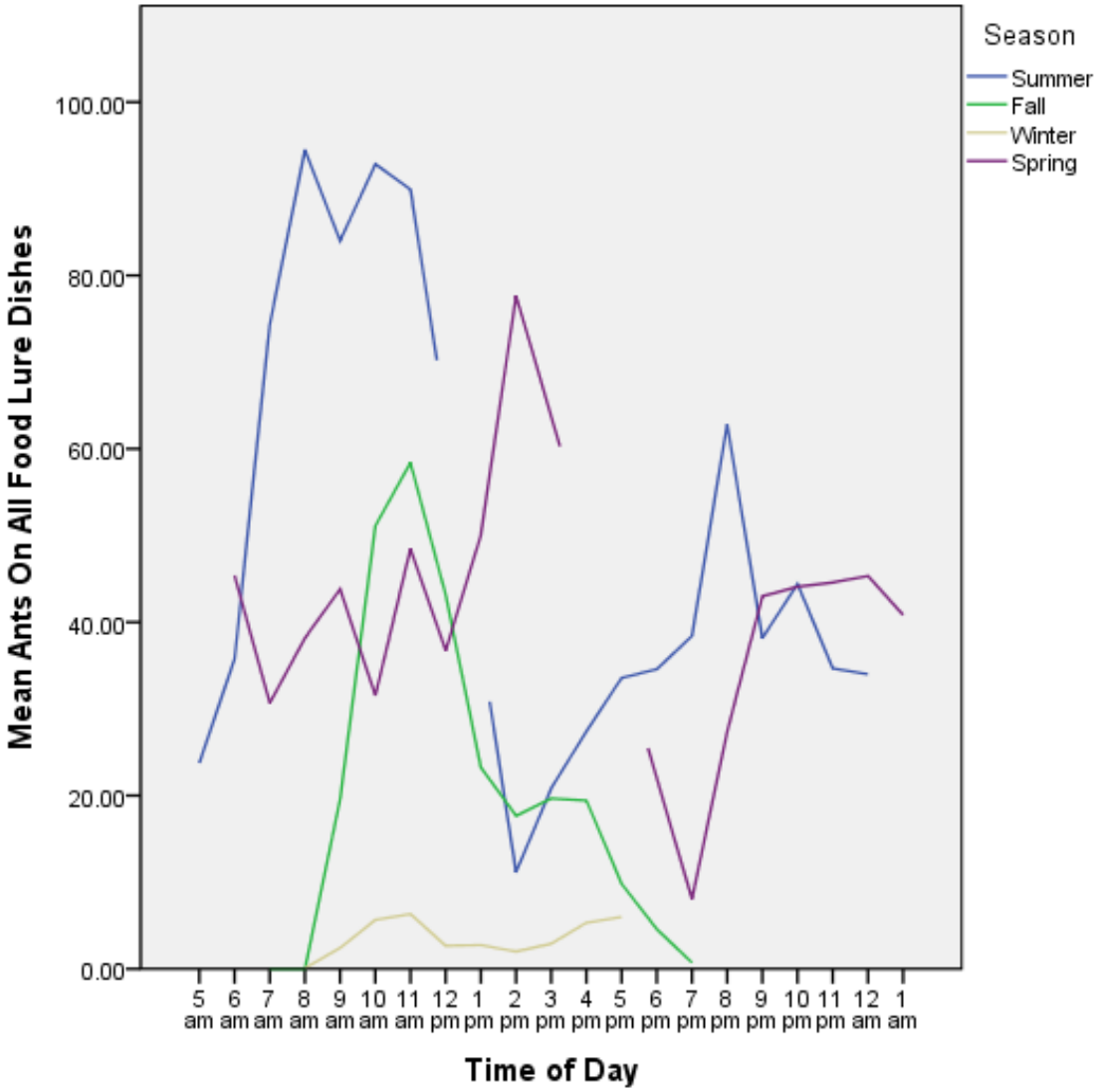


Fig. 22. Peak foraging time of *N. fulva* across all seasons. During the summer observation *N. fulva* foragers tended to be crepuscular, foraging during the early morning and late afternoon hours. During all other seasons ants were seen foraging closer to the middle of the day, when temperatures were between the foraging thresholds.

Conclusions

Food lure preference trial data showed that *N. fulva* were attracted to carbohydrate rich food lures (MAJ and HW) or HD, a moderately protein based food lure (Table 3). The switching of food lure preference with cover-type suggests that consistency of food lures may determine acceptability. MAJ tends to liquefy in the sun, and HD desiccates in direct sunlight. This may explain the preference switch for MAJ in the sun and HD in the shade. Yet, the opposite was true in the spring with MAJ preferred in the shade and HD in the sun. This unexpected shift suggests that there are variations in nutritional needs within a colony; or that *N. fulva* colonies prefer collecting solid versus liquid foods during different seasons. However, the diet preference trials always revealed a preference for the most carbohydrate rich diet.

MAJ and HD were both acceptable food lures when assessing *N. fulva* relative abundance based on relative abundance and consistency across seasons. Ease of use should be considered when deciding the most appropriate lure. HD are often cut into 4 mm slices and placed on the ground with a flag skewered through the middle for ease of location and collection. Another methodology employed is placing a 2mm slice of HD on a white tile and taking a picture of attracted ants after a defined recruitment period (Drees et al. 2010b). MAJ and HD can both be put into a small test tube and placed on the ground or attached to surfaces such as plant stems, trunks, or limbs. A pairing of these two complimentary food sources would provide a sampling technique appropriate for all seasons and cover-types.

In comparison to *N. fulva* preferences, *Linepithema humile* workers assayed in California (season not provided) preferred a diet of 25% honey water solution to undiluted honey, tuna meal plus honey, corn meal plus yeast and honey, tuna meal, and corn meal plus yeast (Baker et al. 1985). Tuna, the Xstinguish^{TM} (Bait Technology Ltd., New Zealand) (a protein based ant bait) matrix, and sugar water were the most attractive baits to *P. longicornis*, and preference did not shift between seasons (Summer and Fall) (Stanley and Robinson 2007). However, Stanley and Robinson (2007) suggested that tuna was economically impractical for management programs. Phenology of brood and alate production accounts for much of the interspecific differences between diet preferences.

Much can be gleaned from attractiveness, or lack thereof, of the other lures utilized in this study. *Nylanderia fulva* were attracted to the scrambled chicken egg, especially in the fall, but much less so in the spring (Table 4). This could indicate that *N. fulva* may have ecological implications with respects to predation on pipped eggs of birds..

Nylanderia fulva were not attracted to vegetable oil in any season. Fats alone, as opposed to those mixed with protein, are probably not needed by *N. fulva*. Traditional bait matrices utilize defatted corn grit with soybean oil as an attractant. Unfortunately, *N. fulva* workers, much like other crazy ant species (Stanley and Robinson 2007), are not attracted to corn grit with vegetable oil-based bait products (Drees et al. 2010a). The unattractiveness of oils to *N. fulva* may explain the ineffectiveness of fire ant baits against these ants.

Group transport of food was seldom observed and, when encountered, appeared to be poorly executed. Even so, the mealworm was occasionally dragged out of the foraging trays.

Cook et al. (2011) determined that an optimal laboratory diet for *N. fulva* consisted of a p:c ratio of 1:2, but hypothesized that there would be a seasonal shift for diet preference in the field. Cannon and Fell (2002) reported similar results (1p:2.3c) through all seasons for *Camponotus pennylvanicus*. Furthermore, *Nylanderia guatemalensis* was determined to prefer a carbohydrate rich diet (Hahn and Wheeler 2002). This diet preference experiment confirmed that *N. fulva* preferred a carbohydrate rich diet through all seasons in the field, but no seasonal shift was detected for diets used. Although diet preferences were detectable in three seasons, overall foraging on diets was minimal. The dry nature of the artificial diet may have negatively influenced diet acceptability because formicine ants generally prefer liquid foods (Cook et al. 2011). When the same diets are offered to *N. fulva* in laboratory colonies, attractiveness was minimal. It is possible that *N. fulva* would more readily feed on diets with higher moisture contents (Cook et al. 2011).

Preference of foragers for one food over another may not only reflect seasonal requirements of colonies for nutrients contained in preferred foods, but may also be an indication of the relative availability of nutrients in the environment (Stein et al. 1990, Hahn and Wheeler 2002, Kay 2004). Arboreal ants typically prefer protein-based baits (Kaspari and Yanoviak 2001) whereas terrestrial ants typically prefer carbohydrate-based baits (Hahn and Wheeler 2002). In addition, preference can be a product of

nutrient limitations in a species territory (Kay 2004), with proteins being limiting for arboreal ants, and carbohydrates resources limiting for terrestrial ants (Hahn and Wheeler 2002). *Nylanderia fulva* seem to be mostly terrestrial but have been observed foraging and even carrying brood up trees. Being polydomous may shorten the length traveled between food sources and a nest (nutrient storage facility), thereby enhancing foraging efficiency (Hölldobler and Wilson 1990). Many of the trees in the East Columbia, TX field site were pecans, *Carya illinoinensis*. *Nylanderia fulva*, being exudate feeders, may be attracted to pecan aphids such as *Melanocallis caryaefoliae* or *Monelliopsis pecanis*. Because *N. fulva* have a strong preference for carbohydrates, this is most likely the limiting nutrient for *N. fulva* in the field which would be more characteristic of terrestrial species. However, arboreal ants tend to be a more aggressive and numerically dominant species than terrestrial ant species (Hahn and Wheeler 2002); which may be a product of their high carbohydrate diets (Grover et al. 2007), and explains in part the aggressiveness and ecological success of *N. fulva*.

The rapid discovery and subsequent domination of food by *N. fulva* is undoubtedly a key component to their invasive success. *Nylanderia melanderi* was predicted to discover food quicker than *Prenolepis imparis* or *Aphaenogaster rudis* because of their rapid rate of movement (Lynch et al. 1980). Rapid movement also helps *Nylanderia melanderi* to escape when confronted by other ants. *N. melanderi* have small colonies, 125-150 workers, and are a much more timid species than *P. imparis* or *A. rudis* (Lynch et al. 1980). However, *Nylanderia* have a "spray and retreat" defensive tactic wherein they utilize their ability to use formic acid as a chemical projectile

towards an enemy, and then quickly flee before a physical altercation occurs. Unlike *N. melanderi*, *N. fulva* exhibit high population densities. These behavioral advantages allow *N. fulva* to discover food sources quickly and defend them from other species. *Nylanderia fulva* were even observed extirpating RIFA from diets within 1 hour of discovery during this experiment.

Nylanderia fulva forage between 9.95 and 37.26°C with peak foraging activity at 28.24 ± 3.12°C. Of the species ranges listed in Hölldobler and Wilson (1990), *N. fulva* temperature thresholds most closely resemble that of *Tapinoma sessile* (6 to 35°C) (Bernstein 1979), *Aphaenogaster senilis*, (10 to 35°C), and *Crematogaster scutellaris* (11 and 40°C). Provided that foraging thresholds are any indication of geographic range tolerance, this could be used as a factor in future prediction models for *N. fulva*. Invasive *Nylanderia* species currently occupy much of the gulf coast and are likely to be closely confined to these areas by temperature and humidity levels. Although *L. humile* prefers a cooler foraging range (5 to 35°C) they are behaviorally similar to *N. fulva* and occupy much of southern California and the Gulf Coast states.

It is not uncommon for ants to exhibit diel foraging. *Formica rufa* (Skinner 1980), *L. humile* (Markin 1970) and *Anoplolepsis longipes* (Rao and Veeresh 1991a) exhibit fluctuating foraging intensities throughout the day and night. *Nylanderia fulva* exploited a diel foraging schedule in the spring and summer. This is probably due to temperatures within the tolerable thresholds, and therefore, *N. fulva* may exhibit diel foraging in all seasons on days when temperatures are favorable. Although general

trends were observed when looking at the time of day of foraging peaks between seasons, temperature should prove to be more accurate at predicting *N. fulva* activity.

Cook et al. (2011) determined that *N. fulva* was not capable of collecting large amounts of food to compensate for deficits in limiting nutrients. The geometric framework commonly utilized for diet preference studies provides studies in nutrition with an experimental estimation of the optimal intake, nutrient, and growth targets of individuals or a population (Simpson and Raubenheimer 1995). These experiments often determine optimal blends of nutrients over the life of an insect (Simpson and Raubenheimer 1996) and show how ants select among diets of differing nutrient concentrations to satisfy an optimal nutrient intake target (Dussutour and Simpson 2008a). However, when determining initial diet preference to subsequently develop monitoring and baiting techniques, knowledge of nutrient targets regarding optimal performance of an individual or population is unnecessary.

Ant preference for proteins, carbohydrates, lipids, particle size, and the seasonal variation between these preferences will determine the appropriate matrices for toxic baits used against a targeted species. Food attractants in toxic baits must act as a solvent for the toxicant, without deleterious effects, and survive several weeks of storage while remaining acceptable to the target species (Banks 1990). The results of this study suggest that matrices for *N. fulva* should include ample amounts of carbohydrates, have high moisture contents, and should not utilize oils for the infusion of active ingredients. Sugar water solutions impregnated with toxicants such as in Greenberg et al. (2006) may

provide adequately attractive and efficacious baits as long as they are used in large enough quantities for the enormous biomasses of *N. fulva* populations.

CHAPTER V

FECUNDITY OF *Nylanderia fulva* UNDER LABORATORY CONDITIONS (HYMENOPTERA: FORMICIDAE)

Introduction

The extreme polygynous nature of *N. fulva* has undoubtedly been a major factor in the invasive success of this species in southeast Texas. Evaluating the reproductive potential of *N. fulva* is a vital step in identifying the biological idiosyncrasies and invasive potential of the species. Such understanding will allow for population estimations.

Semantics play an influential role in the terminology regarding reproductive potential. "Fecundity" is the number of offspring produced by an individual insect, whereas "fertility" refers to the number of eggs that hatch (Awmack and Leather 2002). "Potential fecundity" refers to the number of eggs within the reproductive tract, whereas "achieved" or "realized fecundity" is the number of offspring actually produced during the lifetime of an individual (Awmack and Leather 2002). Although potential fecundity may be a good indication of theoretical, future reproductive output, the difference between potential and achieved fecundity can be substantial as a result of variation in factors such as nutritional quality, environmental conditions, and population density (Coulson 2009). "Realized natality" is the actual production of new individuals under a given set of environmental conditions (Coulson 2009). Although this definition fits well with the discussion of egg production, the ambiguity related to whether or not "new

individuals" refers to eggs or adults is problematic. For the purposes of this study, fecundity will be used to describe the observed level of reproductive performance.

The rate of egg production can be manipulated based on the conditions of the colony (Hölldobler and Wilson 1990). Several factors influence the fecundity of individual queens including: nutrition quality, temperature, humidity, photoperiod, queen weight, worker to queen ratio, quantity and age of brood, and the number of queens per colony (Arcila et al. 2002a, Hölldobler and Wilson 1990, Tschinkel 1988, Wilson 1974). Furthermore, egg production is species specific in ants and has been documented to range from 400/queen/yr. in *Myrmica rubra* (Schneirla 1971) to 2,000,000/queen/yr. in *Eciton burchelli* (Hölldobler and Wilson 1990).

Wilson (1974) suggest that in *Plagiolepsis pygmaea* and *Leptothorax curvispinosus*, the number of workers, rather than the number of queens, is the prime determinant of the total fertility rate and subsequent potential for colony growth (Holdobler and Wilson 1990). This can be explained by the increased rate of queen feeding by workers (Arcila et al. 2002a); however, Tschinkel (1988) determined that in *Solenopsis invicta*, workers alone do not control monogyne queen fecundity, but that the presence of 4^{th} instar larvae caused the egg laying rate of queens to surge. This is also evident by ants feeding the queen more when final-instar larvae are present (Hölldobler and Wilson 1990). Polygynous *S. invicta* colonies have higher brood to worker ratios than monogynous colonies, which suggests that the number of queens can also drive individual fecundity (Hölldobler and Wilson 1990).

According to Hölldobler and Wilson (1990) queens in polygyne colonies should contribute fewer offspring than they would if they were the sole egg-layers. Aggression and dominance between queens in polygynous colonies can affect fecundity through periodic attack (Wilson and Brown 1984), dominance posturing (Evesham 1984), eating of rival eggs (Fowler and Roberts 1983), and the use of pheromones to suppress ovarian development (Hölldobler and Wilson 1990). According to Arcila et al. (2002a) laboratory study of *N. fulva* in Columbia, monogyne colonies produced more eggs than polygyne colonies, yet polygynous colonies of *N. fulva* were more successful in rearing brood to the pupal stage than monogyne colonies (Arcila et al. 2002b).

Not all species of *Nylanderia* are polygynous. *Nylanderia flavipes, Nylanderia melanderi* and *Nylanderia pallida* are monogynous and have very small colonies (100, 105 and 500 workers respectively) relative to *N. fulva* (Ichinose 1986, Lynch et al. 1980). The lifespan of *N. flavipes* workers, including immature stages, is estimated to be 2 years (Ichinose 1987a).

Nylanderia flavipes alates overwinter in their parent colonies and have nuptial flights in late June to early July of the following year (Ichinose 1987a). *Nylanderia flavipes* workers rear fewer sexual larvae in the presence of their mother queen (Ichinose 1994). However, Passera (1994) suggests that *Nylanderia* mating occurs inside the nest or at its entrance, resulting in absence of nuptial flights (Solis et al. 2007). This appears to be the case for *N. fulva*. Males of *N. fulva* are commonly found throughout the year in Texas, as well as in Columbia (Arcila et al. 2002b), but mating flights have not been reported. Pearcy et al. (2011) suggested that sibling mating without inbreeding in *P. longicornis*,

is an important pre-adaptation for the crazy ant's invasive success. This is achieved as a result of queens being clones of their mothers, whereas males do not inherit maternal alleles and are genetically identical to their fathers. Small colonies can repeatedly mate without detrimental phenotypes caused by deleterious recessive alleles. This may be occurring in populations of *N. fulva*, and may also contribute to their invasive success.

Nylanderia fulva and *P. longicornis* workers have three larval instars while *N. flavipes* and male *N. fulva* have 4 (Arcila et al. 2002b, Fox et al. 2007, Ichinose 1987b, Solis et al. 2007). The mean duration of *N. fulva* egg, larva, and pupa development was 16.2, 10.8, and 12.2 days, respectively with a total immature development time of 23-50 days and a mean of 39.2 days (Arcila et al. 2002b, Gomez et al. 2002). *Paratrechina longicornis* has a mean duration of egg, larva, and pupa development of 16.1, 18.3, and 12.3 days, respectively (Solis et al. 2007).

Immature developmental times of *Anoplolepis longipes*, *Camponotus* sp., and *Prenolepis imparis* are 59.6, 48-74, and 70-90 days, respectively (Rao and Veeresh 1991b, Hölldobler and Wilson 1990). Queen *A. longipes* lay 7-22 eggs/day (Rao and Veeresh 1991b). The mean duration of *A. longipes* egg, larva, and pupa development is 18.6, 19.4, and 21.6 days, respectively with a total immature development time of 59.6 days for workers. The longevity of workers, males, and queens is 94.3, 10.5, and 137.5 days, respectively and workers make up about 98% of the total biomass of the colony.

Protein availability is known to influence reproductive decisions in eusocial Hymenoptera (Aron et al. 2001). *Linepithema humile* was shown to increase the number and size of sexual pupae produced with protein supplementation in the laboratory (Aron

et al. 2001). The number of workers produced was unaffected, but they also increased in size with an increase in available protein. In social insects, the size, caste, and reproductive capacity of adults is also determined in part by nutrition during larval development (Cassill and Tschinkel 1995, Wheeler 1994).

Little is known about the biology of *N. fulva* in Texas. Previous studies focused on identification, distribution, and management (Meyers 2008). This experiment is the second investigation of *N. fulva* fecundity and the first fecundity experiment within the United States. This experiment was intended to close the information gap on the biology of *N. fulva* in Texas.

Materials and Methods

Queens and workers were collected from 30 colonies (nesting sites including workers, alates, and brood) ≥ 10 m apart in April of 2012 from East Columbia, TX. Colonies were transported to the Center for Urban and Structural Entomology and extracted from soil and leaf litter using the modified drip technique (see chapter 2). Voucher specimens from this study were deposited in the Center for Urban and Structural Entomology and Texas A&M University Insect Collection (Voucher #688), Department of Entomology, Texas A&M University, College Station, Texas.

Several factors influence the fecundity of individual queens: nutrition quality, temperature, humidity, photoperiod, weight, and the number of queens per colony (Arcila et al. 2002a). During experimentation, all of these conditions were kept constant except for the number of queens per colony, the independent variable. Using a design by

Arcila et al. (2002a), experimental colonoids (experimental colonies separated from their parent colonies) consisted of 10 replicates of one queen (monogynous), three queens (trigynous), and six queens (hexagynous) with 100 workers per queen. Controls consisted of 10 replicates of 100 workers and no queens to insure that workers were not producing eggs and that no eggs were being introduced to colonies during aspiration and transfer to arenas. Queens and workers were placed in 14 cm Petri dishes. The vertical walls of each Petri dish were coated with Insect-a-Slip (BioQuip Products, Inc.) to prevent escape, and the bottoms were painted with Rust-Oleum® Specialty Plastic black spray paint for ease of observing eggs produced. Colonies were provisioned with water, 10% honey-water, 0.5 g of Bar S Hot Dog, and 2 dead crickets. For harborage, a 5.5 cm Petri dish was filled 1/3 full with dental stone and dampened with water to provide moisture. The lid was painted black with Rust-Oleum® Specialty Plastic black spray paint to provide seclusion, and a 7 mm hole was burned into the top of the lid for access. Colonies were kept at 27°C and 80% R.H. with a 14:10 light to dark photoperiod. The colonies were allowed to function normally for three days, after which time, the colonies were placed into a -20C freezer for 12 hrs to terminate egg production. Eggs were counted using a Nikon SMZ-2T microscope. All 100 queens were retrieved and weighed using an A&D Electronic Balance. Worker egg load was determined by counting the number of eggs in a clutch grasped by a single worker. A general linear model was used to compare means and determine the correlation between weight and egg production.

Results

As queen number increased, individual fecundity increased (Fig. 23). However, statistical significance was not achieved ($p = 0.06$) possibly due to 80% of monogyne colonies not producing any eggs, which resulted in a large standard error for that treatment (Fig. 24). Mean egg production per queen for monogyne, trigyne, and hexagyne colonies were 0.11 ± 0.25 eggs/hr, 0.19 ± 0.09 eggs/hr., and 0.25 ± 0.12 eggs per hour. No eggs were produced in the control group.

As the number of queens per colony increased, the number of eggs produced per colony increased (Fig. 25). The average queen weight was 3.65 ± 0.39 mg, but no significant relationship was found between queen weight and individual fecundity (Fig. 26).

Worker egg load, the mean number of eggs carried by a single worker, was 31.97 ± 21.06; however, the largest egg cluster found consisted of 113 eggs. It is unknown whether this worker was actually carrying that egg cluster, or if she attached to the cluster as she was dying. Therefore, removing this outlier, the corrected mean worker egg load was 29.52 ± 15.68 eggs.

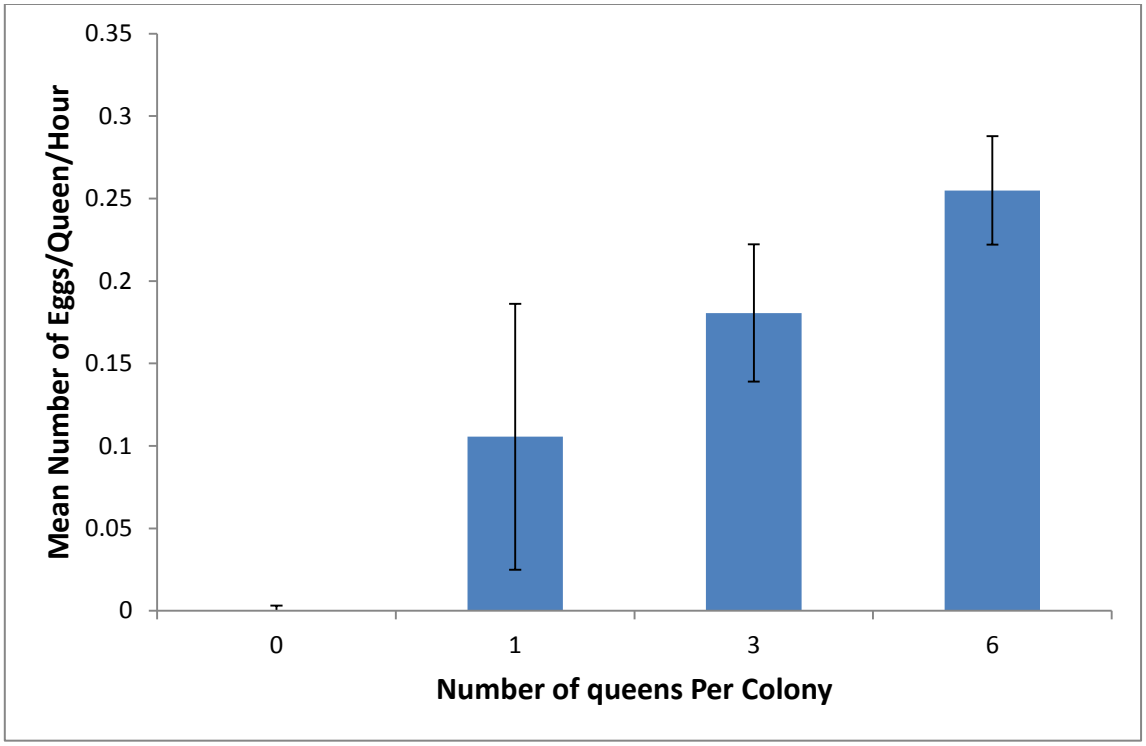


Fig. 23. Queen number effects on individual fecundity. As queen number increased, individual fecundity increased. $p = 0.06$ ($\alpha = 0.05$).

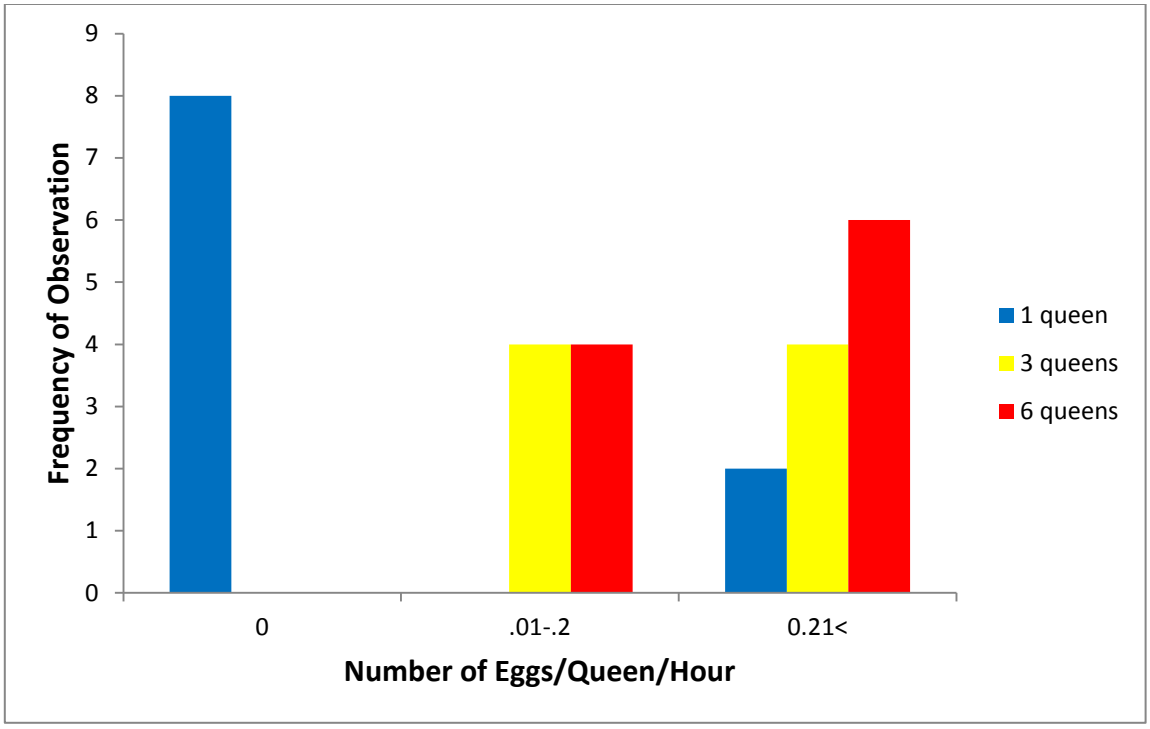


Fig. 24. The frequency of observations within different ranges of fecundity. Since 80% of monogyne colonies did not produce eggs, a large standard error resulted in figure 1. Subsequent no mean separation was found between treatments.

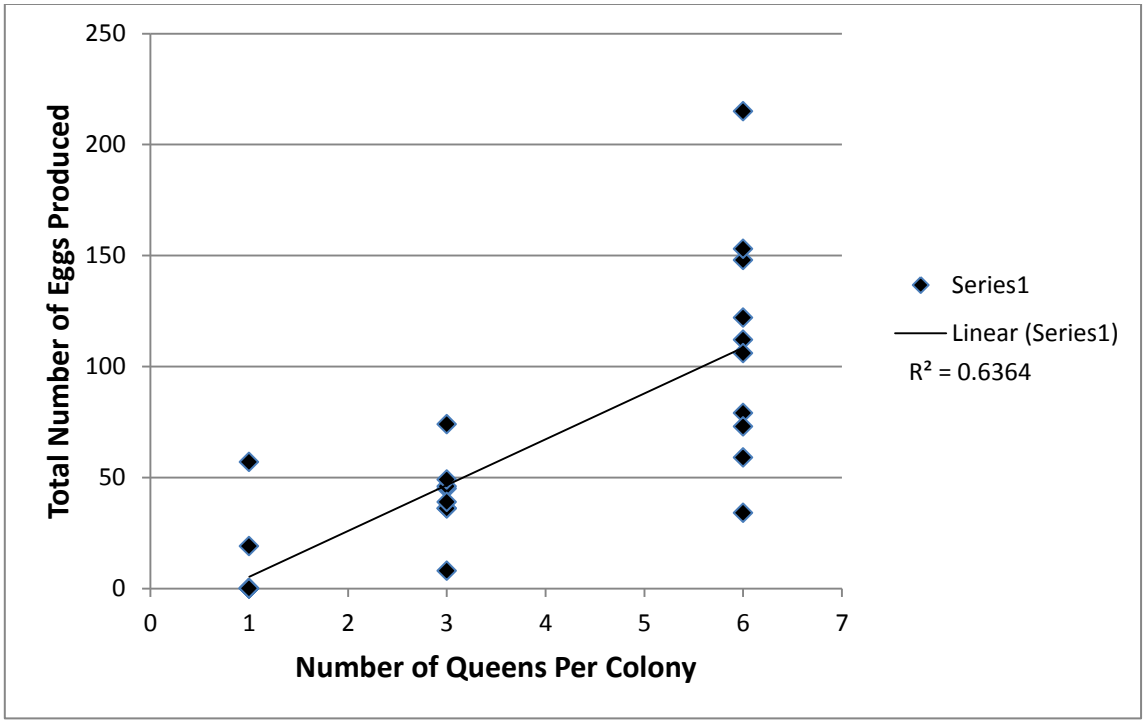


Fig. 25. Effects of the number of queens per colony on total number of eggs produced. There was a significant correlation between the number of queens per colony and the number of eggs produced.

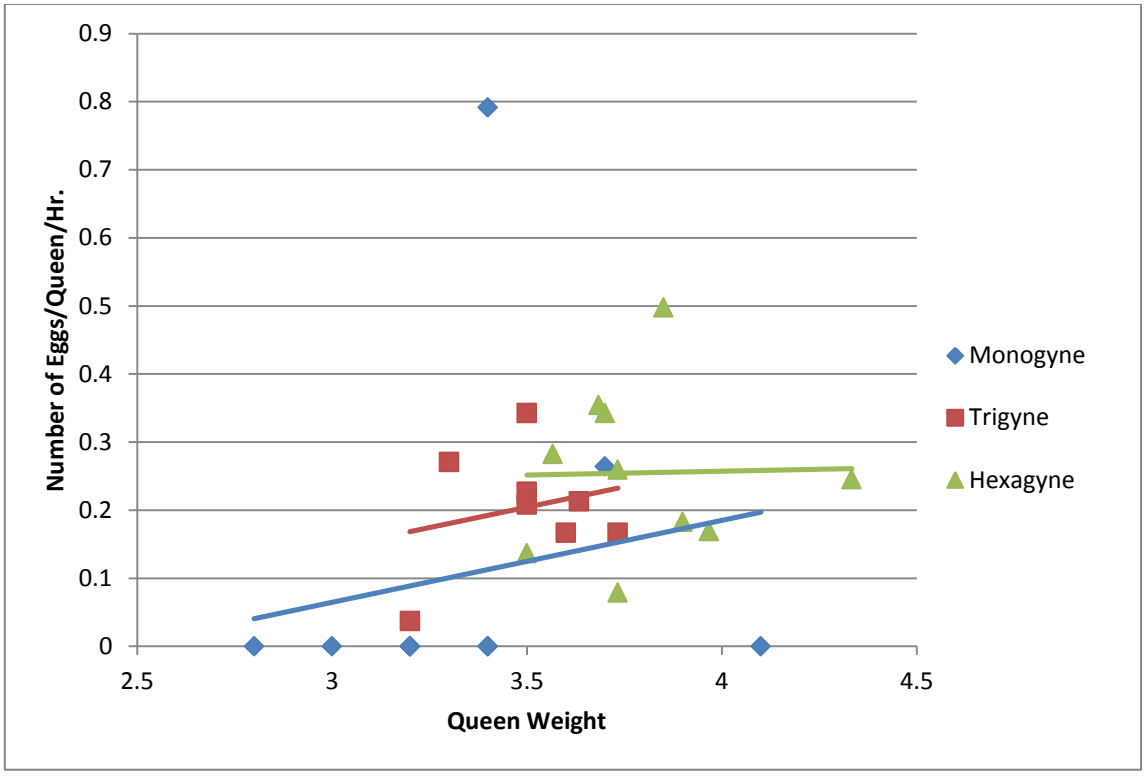


Fig. 26. Effect of queen weight on number of eggs/queen/hr. There was a slight positive relationship between queen weight and individual queen fecundity, yet there was no significant correlation (monogyne $R^2 = 0.0293$, trigyne $R^2 = 0.0557$, and hexagyne $R^2 = 0.0005$).

Conclusions

Arcila et al. (2002a) reported a negative relationship between *N. fulva* queen number per colony and individual oviposition rate. This was the opposite of our findings for *N. fulva* in Texas. As queen numbers increased, individual fecundity increased. In the field, *N. fulva* colonies always consist of multiple queens. Therefore, the hexagyne colony fecundity of 0.25 ± 0.12 eggs/queen/hr should be considered most representative of field application. However, it is common to find between 15-20 queens per colony

under each landscape article, and it is yet to be determined if laboratory colonies with this extreme number of queens would yield a higher production of eggs per queen.

The absence of eggs in the control group suggests that workers do not produce eggs within 3 days of exclusion from queens, and that no eggs were transferred from the parent colony to the colonoids during aspiration.

Egg production was much lower than predicted based on the Arcila et al. (2002a) results. Factors that may have negatively influenced maximum fecundity include the time of year in which the study was conducted (April 2012), environmental conditions, nutritional needs, disruption of circadian rhythms, the effects of aspiration of queens, and the ratio of workers to queens and brood. In the summer of 2009, a non-replicated proof of concept was conducted where one queen was isolated with 10 workers and a food source. She produced 2 eggs per hour. This may have been due to the difference between the seasons in which these studies were conducted. However, as a result of the non-replicated nature of the proof of concept, no causative effect could be determined. Ant species often behave differently in introduced areas than they do in their native ranges (Wilder et al. 2011). Additionally, the 27°C and 80% R.H. with a 14:10 light to dark photoperiod used by Arcila et al. (2002a) may not be optimal for Texas *N. fulva* populations. The inclusion of a vitamin supplement such as the Vanderzant vitamin mixture (Dussutour and Simpson 2008b), may also be necessary for proper nutritional needs. Colonies were collected from the field, transported to the laboratory, extracted from the substrate using a modified drip technique, and separated into colonoids by aspiration. This may have disrupted the normal rate of egg production by queens. The

worker to queen ratio of 100:1 may also have influenced the brood-rearing capacity of the colonoid, which may have influenced queen egg laying rate. An extreme ratio of 1000:1 and the addition of larvae at study initiation are recommended for future experiments. All of these factors should be explored before a definitive maximum fecundity can be determined, but the intent of this study was to be able to directly compare the results to *N. fulva* fecundity using the same parameters as Arcila et al. (2002a).

Since monogyne colonies rarely (20% of the time) produced eggs, perhaps *N. fulva* is close to obligatory polygyne, at least in Texas. That is to say that there is a causative relationship between the presence of multiple queens and an increase in, and initiation of, egg production. The mechanism for this synergistic effect is unidentifiable from this study. I have not found this synergy reported for any other ant species. Polygyne species typically have a dominant queen which suppresses the egg laying rate of other queens through pheromonal inhibition (Hölldobler and Wilson 1990, Tschinkel 2006). Pheromonal inhibition is a strategy for insuring the successful replication of an individual's genes in subsequent generations. The same is true for increasing egg laying rate in the presence of other queens. Workers within the polygyne colonies of *N. fulva* seem to indiscriminately share resources with colony members. Conceivably, individual workers are more predisposed to provide provisions to the closest of kin, optimizing the success of their mother and, subsequently, their genes.

Although, it is possible that unmated, dealated queens were selected for the monogyne colonies, this is unlikely. With 80% of monogyne colonies not producing any

eggs, it would be expected that at least one replicate of trigyne colonies would not produce any eggs, if there was no synergistic effect between queen number and fecundity.

Arcila et al. (2002a) found that there was a significant positive correlation between queen weight and the number of eggs produced, but no cause-effect relationship was found. The results of this study indicate a slight positive relationship between queen weight and the number of eggs produced but there was not a strong correlation.

Worker ants carried an extraordinary number of eggs in their mandibles, making eggs difficult to count while the colony was still alive. This prompted the decision to terminate the colony in order to count eggs. Interestingly, as workers died they maintained control of their egg load. Most eggs and queens were found within the harborage provided. Occasionally a queen was observed outside of the harborage with egg clusters a short distance away.

Fecundity is a measurement of fitness and is paramount in understanding the population ecology of *N. fulva*. Although individual queen fecundity was lower than expected, the extreme polygynous nature of *N. fulva* results in an incredible population density, and may have a synergistic effect with individual queen fecundity. Survivorship of eggs to adulthood is undoubtedly high. Coupled with the development of a life table, knowledge of invasive species fecundity and phenology may lead to integrative management techniques that minimize their reproductive success, such as the application of insect growth regulators before peak egg production occurrences.

CHAPTER VI

MECHANICAL VECTOR POTENTIAL AND MICROBIOTA ASSEMBLAGES OF *Nylanderia fulva* (HYMENOPTERA: FORMICIDAE)

Introduction

The transmission of pathogenic microorganisms by arthropods, whether mechanically or through directly feeding on a host, poses human, animal, and plant health complications throughout the world. Many arthropods are mechanical vectors of pathogenic microorganisms including ants, cockroaches, flies, spiders, and wasps (Daniel et al. 1992, Fotedar et al. 1992, Gliniewicz 2003, Graczyk et al. 2003, Imamura et al. 2003, Lemos et al. 2006). In addition, some ant species such as *Solenopsis invicta* and *Solenopsis saevissima* are considered medically important due to their painful stings that may cause allergic reactions including anaphylactic shock (Wetterer and Snelling 2006). Determining the vector potential, and therefore, medical importance of a species, will emphasize the importance of their management, especially in medically sensitive areas.

Beatson (1972) established that *Monomorium pharaonis* (pharaoh ants) are a mechanical vector of pathogenic microorganisms. He collected ants from nine hospitals and a school of veterinary medicine in Cambridge, England. He isolated *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Streptococcus* spp., and *Clostridium* spp. from ants collected in hospitals and *Bordetella bronchiseptica* from ants in the veterinary hospital. The suggested mechanism for transmission was that

worker ants became contaminated and then traveled across food, wounds, or medical supplies. Beatson recorded *M. pharaonis* trailing at least 21.9 m and visiting bedpans, toilets, sluices, drains, and sinks. Ants were also found on medical equipment such as drip-tubes, resuscitation equipment, and sterile packs. They were reported to attack human patients at wounds, particularly those persons with fever, profuse sweating, and burn wounds. Ants were seen biting infants around the eyelids, cheeks, lips, neck folds, and the backs of ears (Beatson 1972, Eichler 1990). These attacks caused skin irritation and lesions as well as pathogen transmission (Eichler 1990). In veterinary clinics, piglets were attacked when *M. pharaonis* were attracted to the swine nasal mucus.

As many as 32 ant species have been shown to be associated with hospitals, and all have been implicated as potential mechanical vectors of pathogens (Table 10). Most ant species that are known to colonize hospitals are exotic species, unicolonial, polygynous, reproduce by colony fragmentation, have a high reproductive capacity, and are very small (1.3 mm to 2.2 mm) (Bueno and Fowler 1994, Moreira et al. 2005). *Monomorium pharaonis*, *Paratrechina longicornis*, and *Tapinoma melanocephalum* were identified as the species with the most potential as carriers of pathogenic organisms because of their frequency of occurrence in hospitals and their association with microbiota (Bueno and Fowler 1994, Edward and Baker 1981, Fowler et al. 1993, Lise et al. 2006, Moreira et al. 2005, Pantoja et al. 2009).

There have been 62 bacteria species, 43 fungi species, and one virus identified as pathogenic organisms associated with ants (Boursaux-Eude and Gross 2000, Chadee and Le Maître 1990, Daniel et al. 1992, Dos Santos et al. 2009, Eichler 1990, Fowler et al.

Table 10. List of species associated with hospitals and implicated as potential mechanical vectors of pathogenic microorganisms.

Ant Species	Author
Acromyrmex sp.	Dos Santos et al. 2009
Brachymyrmex sp.	Fonseca et al. 2010; Fowler et al. 1993; Lise et al. 2006
Camponotus arboreus	Bueno and Fowler 1994; Fowler et al. 1993; Pantoja et al. 2009
Camponotus melanoticus	Pantoja et al. 2009
Camponotus renggeri	Pantoja et al. 2009
Camponotus rufipes	Bueno and Fowler 1994; Fowler et al. 1993
Camponotus vittatus	Pantoja et al. 2009; Rodovalho et al. 2007
Camponotus sp.	Dos Santos et al. 2009; Fonseca et al. 2010; Lise et al. 2006; Roxo et al. 2010
Conomyrma sp.	Bueno and Fowler 1994; Fowler et al. 1993
Crematogaster sp.	Bueno and Fowler 1994; Fowler et al. 1993
Dorymyrmex sp.	Fonseca et al. 2010
Ectatomma edentatum	Zarzuela et al. 2002
Lasius emarginatus	Daniel et al. 1992
Lasius niger	Daniel et al. 1992
Linepithema humile	Bueno and Fowler 1994; Dos Santos et al. 2009; Fonseca et al. 2010; Fowler et al. 1993; Ipinza-Regla et al. 1981; Zarzuela et al. 2002
Monomorium floricola	Bueno and Fowler 1994; Fowler et al. 1993; Peirera 2008; Zarzuela et al. 2002
Monomorium pharaonis	Bueno and Fowler 1994; Chadee and Maître1990; Edwards and Baker 1981; Fonseca et al. 2010; Fowler et al. 1993; Gliniewicz 2003; Lise et al. 2006
Odontomachus sp.	Dos Santos et al. 2009
Pachycondyla sp.	Zarzuela et al. 2002
Paratrechina longicornis	Bueno and Fowler 1994; Fonseca et al. 2010; Fowler et al. 1993; Lise et al. 2006; Moreira et al. 2005; Pantoja et al. 2009; Roxo et al. 2010; Teixeira et al. 2009; Zarzuela et al. 2002;

Table 10 Continued.

Ant Species	Author
Pheidole aberrans	Zarzuela et al. 2002
Pheidole impressa	Pantoja et al. 2009
Pheidole radoszkowskii	Pantoja et al. 2009
Pheidole sp.	Bueno and Fowler 1994; Dos Santos et al. 2009; Fonseca et al. 2010; Fowler et al. 1993; Pantoja et al. 2009
Solenopsis globularis	Pantoja et al. 2009
Solenopsis molesta	Bueno and Fowler 1994; Chadee and Maître1990; Fowler et al. 1993
Solenopsis saevissima	Lise et al. 2006; Moreira et al. 2005; Pantoja et al. 2009
Solenopsis sp.	Dos Santos et al. 2009; Fonseca et al. 2010; Lise et al. 2006; Pantoja et al. 2009; Roxo et al. 2010
Tapinoma melanocephalum	Bueno and Fowler 1994; Dos Santos et al. 2009; Fowler et al. 1993; Lise et al. 2006; Moreira et al. 2005; Pantoja et al. 2009; Rodovalho et al. 2007; Teixeira et al. 2009
Tapinoma sessile	Chadee and Maître1990; Fonseca et al. 2010
Tetramorium sp.	Zarzuela et al. 2002
Wasmannia auropunctata	Bueno and Fowler 1994; Dos Santos et al. 2009; Fonseca et al. 2010; Fowler et al. 1993

1993, Hughes et al. 1989, Lise et al. 2006, Moreira et al. 2005, Pantoja et al. 2009, Roxo et al. 2010, Teixeira et al. 2009). Most ant transmitted nosocomial infections, those that were acquired within the hospital (Harold and Fowler 2003), were caused by *Micrococcus pyogenes, Proteus vulgaris, Escherichia. coli, Alcaligenes fecalis, Salmonella* sp., *Pseudomonas aeruginosa, Clostridium* sp. and *Bordetella bronchi* (Lise et al. 2006). Some of the pathogenic bacteria associated with ants such as *Acinetobacter* sp., *Enterobacter* sp., *Gemella* sp., *Klebsiella* sp., and *Streptococcus* sp., have also been found to be resistant to antibiotics (Lise et al. 2006, Moreira et al. 2005, Teixeira et al.

2009). Not only do pharaoh ants have the capacity to translocate pathogenic organisms, but bacteria have been shown to remain viable within the digestive tract of the ants, suggesting that frass deposition may also be a source of contamination (Aleskeev et al. 1972). Eichler (1990) suggested that the mechanism for ants acquiring pathogens is the consumption of dead wasps, cockroaches, and *Lucilia* flies.

Studies involving ants and their microbiota assemblages in hospitals have been carried out in Brazil (Bueno and Fowler 1994, Dos Santos et al. 2009, Fonseca et al. 2010, Fowler et al. 1993, Lise et al. 2006, Moreira et al. 2005, Pantoja et al. 2009, Pereira and Ueno 2008, Rodovalho et al. 2007, Roxo et al. 2010, Teixeira et al. 2009, Zarzuela et al. 2002), Chile (Ipinza-Regala et al. 1981), England (Beatson 1972), Czech Republic (Sramova et al. 1992), Poland (Gliniewicz 2003), and Trinidad (Chadee and Le Maître 1990). Ants were found in birth wards, nurseries, pharmacies, operation halls, drug cupboards, intensive care, obstetrics, neurology, and dermatology units (Eichler 1990 Fowler et al. 1993). Beatson (1972) implicated laundry in the translocation of ants between hospitals. Personal belongings brought in by visitors or patients may have also played a role in transportation of ants in hospitals (Moreira et al. 2005).

Much like pharaoh ants, *N. fulva* utilize any available cavity for harborage, and are commonly found associated with human structures during cooler parts of the year. They have been reported in several hospitals and a nursing home within the Houston, Texas medical district by pest control operators. The concern for their potential to mechanically translocate pathogenic microorganisms led to this investigation. This was the first attempt to characterize the microbial community on the exterior of an arthropod

using 454 pyrosequencing, as well as the first study showing the ability of an ant species to deposit novel bacteria.

Materials and Methods

Transfer of Pathogenic Microorganisms

Nylanderia fulva were collected from a site in East Columbia, TX in July 2012. Voucher specimens were deposited in the Center for Urban and Structural Entomology and Texas A&M University Insect Collection (Voucher #688), Department of Entomology, Texas A&M University, College Station, Texas.

Colonies were separated from soil and leaf litter using the modified drip technique. Experimental arenas consisted of two 36 x 30 x 15 cm plastic containers, one designated as the nesting chamber and the other as a food chamber (Fig. 27a). Vertical sides of all containers were coated with Insect-a-Slip (BioQuip Products, Inc.) to prevent escape. Each nesting chamber was provisioned with a half-filled disposable culture tube (Pyrex®) of water, plugged with a cotton stopper, filled to ~3 mm with plaster of Paris on top of the cotton, and vials were wrapped in aluminum foil to provide a dark, humid microenvironment. Via a 15.2 cm length of Clearflo® PVC tubing (0.5 cm inside diameter, 0.8 cm outside diameter, and 0.2 cm wall thickness), the nest chamber was connected to agar dish #1. All tubing was fitted at each end with a #3 rubber stopper with a 0.8 cm hole drilled through the middle. Agar dishes were 10.3 x 8.9 cm cylindrical containers with flat lids (Part # 283C, Pioneer Plastics Inc.). Agar dishes

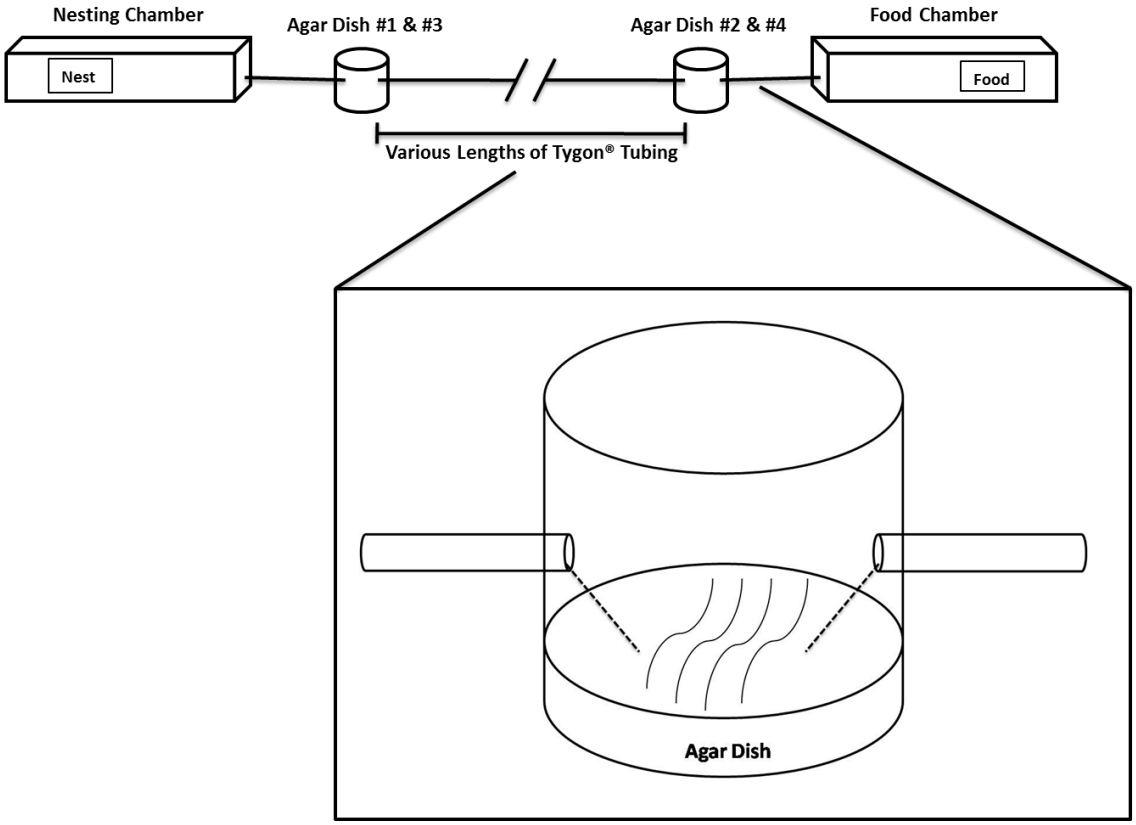


Fig. 27. An illustration showing the various components of experimental arenas. Various foraging distances were simulated by using 3 lengths of Tygon®tubing (1.5 m, 3 m, and 4.5 m).

were filled to 1 cm with LB media (Table 11), over which ants traversed to exit (Fig. 27b). Three transfer distances (1.5 m, 3 m, and 4.5 m) were assessed with six replicates each. The specified length of tubing per replicate (1.5 m, 3 m, or 4.5 m) was used to connect agar dish #1 to agar dish #2. Agar dish #2 was connected to a food chamber via 15.2 cm of tubing. The food chamber contained 10% honey-water solution, a 4 mm hot dog slice, and two crickets. Controls consisted of six replicates of 1.5 m, 3 m, and 4.5 m arenas identical to treatment groups except that no ants were introduced into the arenas.

Table 11. The procedure for making 1 L of LB agar for growing ampicillin resistant *E. coli* used in this experiment.

Constituents	Quantity
Tryptone	10g
Yeast extract	5g
Sodium chloride	10g
Agar	15g
Ampicillin (100 mg/ml)	1ml
Methylparaben (38% in 95% ETOH)	1ml
Water added to 1L	

Experimental replicates consisted of 30 colonoids (experimental colonies separated from their parent colonies) of 250 workers, two queens, and 0.5 g of brood and were added to the nesting chamber. Once ants discovered the food chamber, all agar dishes #1 were replaced with one containing fluorescently labeled *E. coli* lawns (dish #3), and agar dishes #2 were replaced with a sterile agar dish (dish #4). The experimental *E. coli* was a laboratory strain (Top10, Life Technologies, Grand Island, NY) labeled with DsRed-Express protein (Clontech, Mountain View, CA) and an ampicillin resistance gene. The DsRed-Express protein fluoresces when subjected to blue light. The time it took for ants to discover the food chamber was recorded. Agar dishes #1 and #2 were placed in an incubator at 30°C for 48 hours. After incubation the number of fluorescent *E. coli* and fungal colonies were counted, dishes were placed under blue light, and pictures were taken of the plates using a Canon EOS 50D camera with a blue light filter fitted over a 28-135 mm lens.

After ants were allowed to forage throughout the arena for a period of 6 hrs, agar dish #4 was removed and incubated for 48 hrs at 30°C. After incubation, the number of fluorescent *E. coli* and fungal colonies was assessed and a picture was taken as described

above. *Escherichia coli* and fungal colony numbers on plates #1, #2, and #4 were compared statistically via Analysis of variance (ANOVA, α=0.05) (IBM SPSS Statistics 19).

Microbiota Assemblages

Nylanderia fulva were collected from the exterior of one Houston hospital (H1), one Texas City hospital (H2), one Houston retirement community (R1) with a hospital at the center of the complex, and one rural landscape in Pearland (L1) in July of 2012. Ant samples (approximately 50 ants each) were collected using sterilized cotton swabs, placed into 8 ml screw-capped glass vials, transported on dry ice to the laboratory, and stored in a -80°C freezer. Fresh sets of latex gloves were donned at each site to minimize contamination during collection. In order to dislodge microbes from the exterior surfaces of ants, 1 ml of sterile phosphate buffered saline (PBS, 0.01 M phosphate, 0.0027 M KCl, 0.137 M NaCl, pH 7.4) was added to each glass vial and the vials were shaken at 100 rpm for 10 minutes in an incubator at room temperature. The liquid PBS supernatant was aspirated from the glass vials with a micropipette and placed into a sterile 15 ml plastic tube for DNA extraction. Nucleic acids were extracted with a QiaAmp DNA stool mini kit (Qiagen, Valencia CA) according to manufacturer instructions. DNA concentration and purity for each sample was determined with a Nanodrop 1000 UV/Vis spectrophotometer (Thermo Scientific, Wilmingon, DE).

As a quality control measure prior to sending samples off for pyrosequencing on a Roche GS FLX instrument (Molecular Research LP, Shallowater, TX), the DNA

extractions were amplified using conserved primers for bacterial (530F, 1100R) and fungal (nu-SSU-0817-5', nu-SSU-1196-3') ribosomal targets that were previously described (Borneman and Hartin 2000, Dowd et al. 2008). PCR reactions consisted of 2 µl 10X Platinum® *Taq* PCR buffer, 2.4 µl of 25 mM $MgCl_2$, 1.6 µl of a dNTP mixture containing 2.5 mM of each dNTP (Promega, Madison WI), 0.5 µl of 10 µM forward and reverse primer mixture, 0.1 µl of 5 U/µl Platinum® *Taq* DNA polymerase (Life Technologies, Grand Island NY), 1.0 µl of template DNA, and sterile water to a total volume of 20 µl. Thermocycling parameters consisted of 95° C for 10 minutes, then 35 cycles of 95° C for 10 seconds, 55° C for 20 seconds, and 72° C for 30 seconds. PCR reactions were evaluated by agarose gel electrophoresis to confirm the presence of appropriately sized amplicons. PCR amplifications of two extraction blanks (PBS buffer with no ants) served as a buffer/extraction control, and did not produce PCR amplicons with either the bacterial or fungal primer sets.

Ribosomal sequence data was generated using previously described TEFAP methods with Titanium chemistry (Dowd et al. 2008, Dowd et al. 2010). Q25 sequence data derived from sequencing was processed using a proprietary analysis pipeline (Molecular Research LP, Shallowater, TX). Sequences were processed by depleting barcodes and primers, then short sequences < 200bp, sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp were removed. Sequences were denoised and chimeras were then removed. Operational taxonomic units were defined after removal of singleton sequences. Based on the percent query length aligning to database sequence, BLASTn results clustering at 3% divergence (97%

similarity) were classified to the species level, sequences with >3% but <5% were classified to the genus level, >5% but <10% to family level, and >10% but <20% to order level (Dowd et al. 2008, Dowd et al. 2010, Edgar 2010). Operational taxonomic units (OTUs) were taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al. 2006) and compiled into each taxonomic level into both "counts" and "percentage" files. The concentration of template DNA was similar for most samples, but for sample diversity comparisons, results were standardized relative to the starting amount of DNA template.

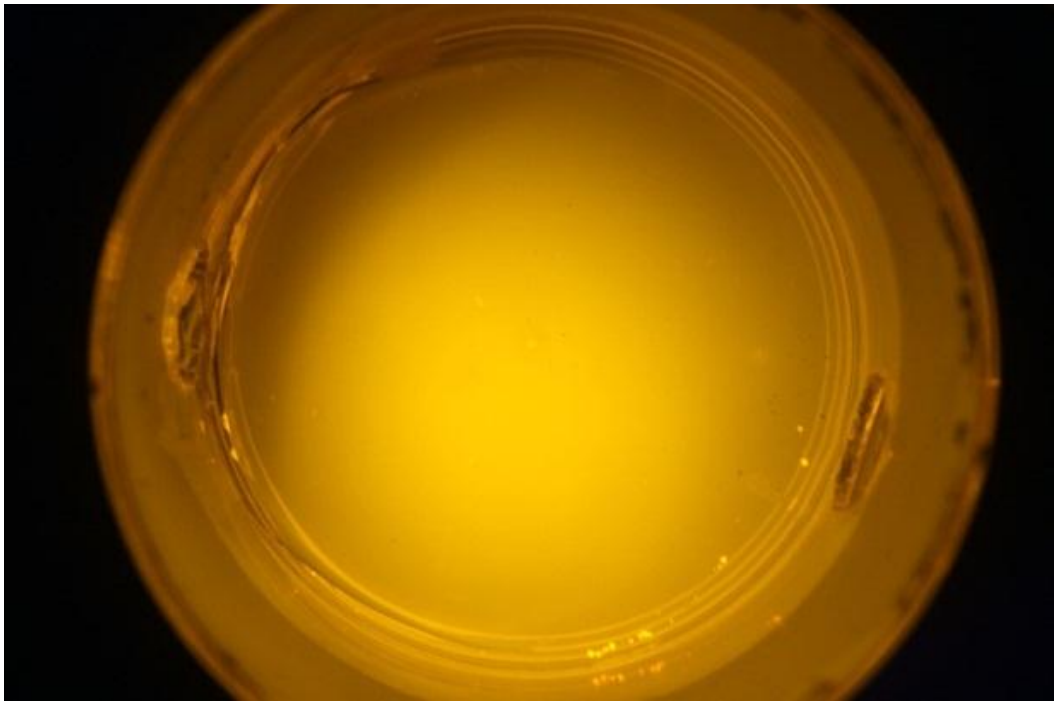
Results

Transfer of Pathogenic Microorganisms

No *E. coli* were found on agar dishes #1 or #2 in treatment or control replicates. However, 77.8% and 38.9% of agar dishes #1 ($p=0.525$, $F=0.657$) and #2 ($p=0.081$, $F=2.718$) respectively contained fungi. The experimental *E. coli* was transferred by *N. fulva* in all treatment groups to dish #4 (Fig. 28a). In the 1.5 m, 3 m, and 4.5 m arenas, 33.3%, 66.6%, and 16.7% of replicates contained fluorescent *E. coli* respectively ($p=0.266$, $F=1.449$), and 83.3% of replicates in all three transfer lengths were contaminated with small colonies of fungi ($p=0.485$, $F=0.759$).

There was no transfer of *E. coli* to any of the control Petri dishes (Fig. 28b). However, there was fungi contamination present in 5.6% of control dishes ($p=0.391$,

a.



b.

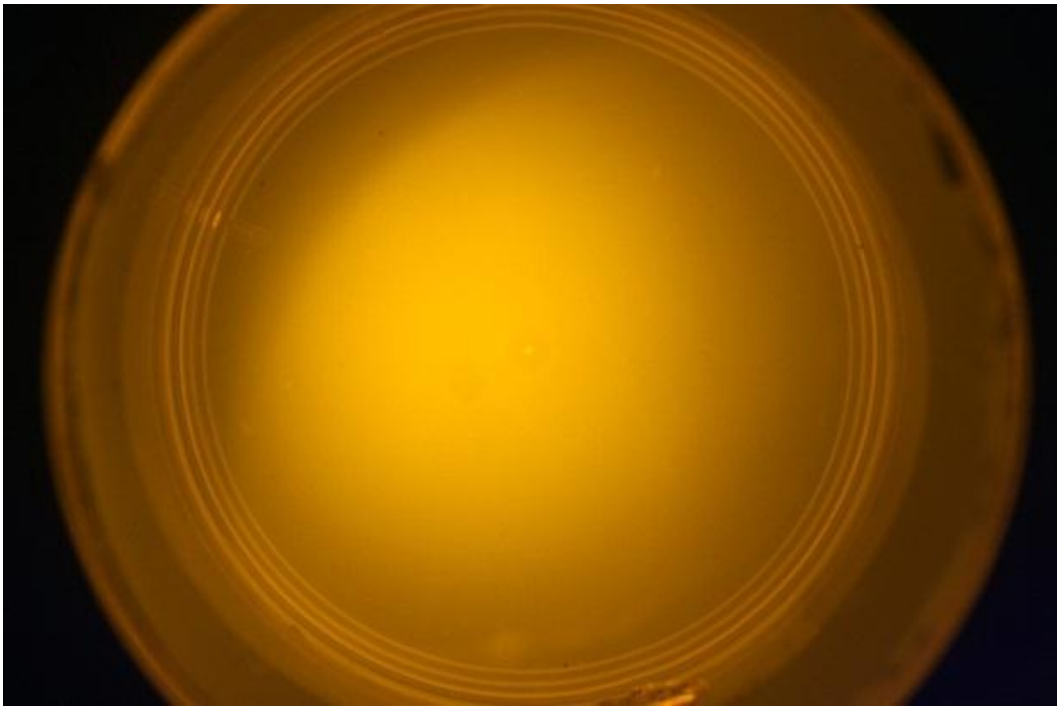


Fig. 28. A comparison of dish #4 treatments (a) and controls (b). A trail of fluorescing *E. coli* (13 individual colonies) can be seen along the bottom portion of photo (a) from the entrance to the exit aperture. A single fungal colony can be seen at the bottom of the control photo.

F=1.0). Fungal contamination consisted of one colony present in each of three dishes. It took an average of 18.3 min/m for ants to discover food chambers.

Microbiota Assemblages

Ribosomal pyrosequencing of bacteria found on the exterior of *N. fulva* generated sequences with homology to 518 different bacterial species (Appendix A). Of those identified, 97 species (18.7%) were potentially pathogenic to either humans, other animals, or plants (Fig. 29). There were sequences with homology to 249, 152, 171, and 327 bacterial species from H1, H2, R1, and L1, respectively. As many as 53 bacterial species were detected on all samples including *Klebsiella oxytoca*, *Serratia marcescens*, and *Streptococcus bovis*. Table 12 provides a breakdown of species occurrence for each collection site.

There were sequences with homology to 135 species of fungi found on *N. fulva* samples in this study (Appendix B). There were sequences with homology to 52, 34, 19, and 86 fungal species from H1, H2, R1, and L1, respectively. There were 61 fungi species (45.2%) that were identified as potentially pathogenic to either humans, other animals, or plants (Fig. 30). Seven species were detected on all samples including *Candida* sp, *Verticillium* sp., and *Fusarium oxysporum*. Table 13 provides a synopsis of species occurrence for each collection site.

Table 14 and 15 shows the results of standardized bacterial and fungal OTUs relative to the amount of template DNA used for pyrosequencing. The bacterial diversity (number of OTUs) was highest in L1 (51.1 OTUs/ ng/μl) and lowest in H1 (4.3

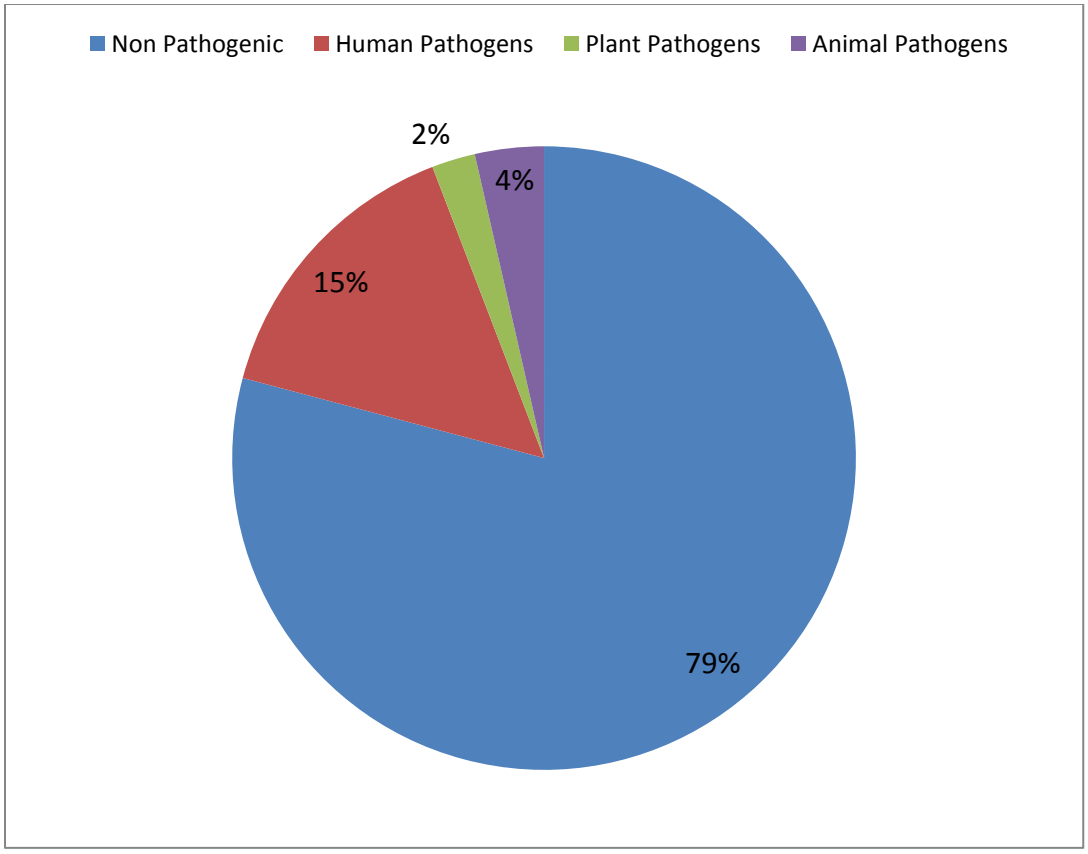


Fig. 29. Bacterial species found on *N. fulva*. Results include data from two hospitals, one retirement community, and one rural landscape (n=518).

Table 12. Bacterial species occurrence for each collection site. Percentages represent either the percentage of the total number of species or the percentage of the total number of sequences.

	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Total number of sequences	58395	5661	13760	14097
Total number of species	249	152	171	327
Pathogenic species	66 (26.5%)	51 (33.6%)	59 (34.5%)	69 (21.1%)
Human pathogenic species	41 (16.5%)	36 (23.7%)	42 (24.6%)	50 (15.3%)
Human pathogen sequences	17901 (30.7%)	1713 (12.1%)	980 (17.3%)	2456 (17.8%)
Plant pathogenic species	11 (4.4%)	6 (3.9%)	6 (3.5%)	8 (2.4%)
Plant pathogen sequences	1913 (3.3%)	408 (2.9%)	160 (2.8%)	262 (1.9%)
Animal pathogenic species	14 (5.6%)	9 (5.9%)	11 (6.4%)	11 (3.4%)
Animal pathogen sequences	5280 (9%)	506 (3.6%)	277 (4.9%)	625 (4.5%)

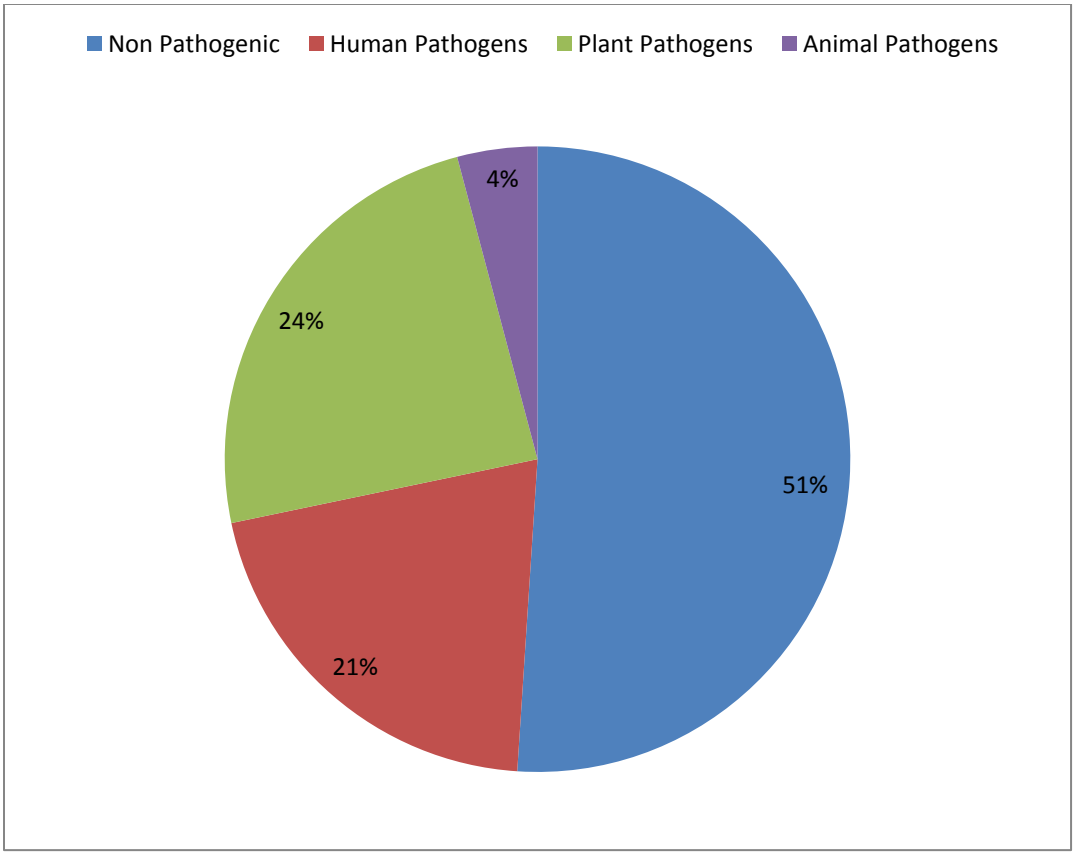


Fig. 30 Fungal species found on *N. fulva*. Results include data from two hospitals, one retirement community, and one rural landscape (n=135).

Table 13. Fungal species occurrence for each collection site. Percentages represent either the percentage of the total number of species or the percentage of the total number of sequences.

	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Total number of sequences	55801	31736	4160	11810
Total number of species	52	34	19	86
Pathogenic species	26 (50%)	12 (35.3%)	8 (42.1%)	47 (54.7%)
Human pathogenic species	15 (28.8%)	3 (8.8%)	2 (10.5%)	18 (20.9%)
Human pathogen sequences	5396 (9.7%)	8016 (25.3%)	759 (18.2%)	3256 (27.6%)
Plant pathogenic species	11 (21.1%)	8 (23.5%)	6 (31.6%)	24 (27.9%)
Plant pathogen sequences	2694 (4.8%)	7026 (22.1%)	485 (11.7%)	2865 (24.3%)
Animal pathogenic species	0 (0%)	1 (2.9%)	0 (0%)	5 (5.8%)
Animal pathogen sequences	0 (0%)	7 (0.02%)	0 (0%)	398 (3.4%)

Table 14. The ectobacterial diversity (number of operational taxonomic units) of *N. fulva*. OTUs = taxonomic units/template DNA (in ng/μl).

	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Template DNA (ng/μl)	59.3	5.4	5.2	6.5
OTUs	4.27	28.70	33.46	51.08
Total Pathogenic OTUs	1.40	9.81	11.54	11.08
Human Pathogenic OTUs	0.91	6.85	8.27	8.00
Plant Pathogenic OTUs	0.20	1.30	1.35	1.38
Animal Pathogenic OTUs	0.29	1.67	1.92	1.69

Table 15. The ectofungal diversity (number of operational taxonomic units) of *N. fulva*. OTUs = taxonomic units/template DNA (in ng/μl).

	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Template DNA (ng/μl)	59.3	5.4	5.2	6.5
OTUs	0.91	6.48	3.85	13.85
Total Pathogenic OTUs	0.44	2.22	1.54	7.38
Human Pathogenic OTUs	0.25	0.56	0.38	2.77
Plant Pathogenic OTUs	0.19	1.48	1.15	3.85
Animal Pathogenic OTUs	0.00	0.19	0.00	0.77

OTUs/ ng/μl); however, the diversity of pathogenic bacteria from R1 (11.5 OTUs/ ng/μl) was slightly higher than L1 (11.1 OTUs/ ng/μl). Overall fungal diversity was highest from L1 samples as well as pathogenic fungal species diversity.

Conclusions

Many arthropods have been determined to be mechanical vectors of pathogenic microorganisms including ants, cockroaches, flies, spiders, and wasps (Daniel et al.

1992, Fotedar et al. 1992, Frishman and Alcamo 1977, Gliniewicz 2003, Graczyk et al. 2003, Imamura et al. 2003, Lemos et al. 2006). Insect invasions are therefore problematic in regards to hospital hygiene. Furthermore, *N. fulva* utilize plants for food sources, tending hemipterans and drinking from floral and extrafloral nectaries, and harborage. This species often ascends large trees with brood, suggesting that they may be moving their nests to utilize cavities created by other animals. They have also been discovered utilizing ears of corn for queen and brood harborage (Paul Nester, personal communication). This close association with trees and food crops presents the possibility of the translocation of plant pathogens.

These results reveal that *N. fulva* are capable of transferring *E. coli* up to 4.5 m in 6 hrs after acquisition from a contaminated source. These ants may be capable of transferring novel microorganisms further distances than this experiment investigated. The maximum foraging range of a colony of *N. fulva* is unknown at this time, but it is plausible that microbiota could be transferred up to that maximum distance. *Nylanderia fulva* may also be capable of transference within a shorter time frame than 6 hrs. The minimal concentration for the acquisition of novel microorganisms is unknown at this time, and should be a consideration in forthcoming investigations.

The absence of *E. coli* in control arenas suggests that bacteria were only transferred in the presence of ants, implicating *N. fulva* as the vector of *E. coli* within the arenas. The minimal amount of fungal contamination in control arenas (5.6%) suggests that airborne fungal spores were present in the laboratory and capable of contamination of agar dishes without the presence of ants. However, the 83.3% occurrence of fungal

contamination on treatment dishes suggests that *N. fulva* were also transferring fungal spores.

It should be noted that because the BLASTn results identified bacterial and fungal species with 97% similarity, false positives were possible. Furthermore, the identification of a species that has been known to be pathogenic does not necessarily mean that the strain of microorganism species on these samples is pathogenic. The 16S ribosomal DNA used in this assay is commonly used to identify bacterial species (Dowd et al. 2008), as is the 182 ribosomal DNA locus used to identify fungal species (Dowd et al. 2010); however, these assays provide no genetic information outside of the ribosomal locus. Therefore, the species identified as pathogenic within this study should be considered "potentially pathogenic".

Although ants could not be collected from the interior of hospitals, microbiota assemblages differed from that of rural landscapes, including additional pathogenic microorganisms. There were 53 species of bacteria and 7 species of fungi that were common to all samples of *N. fulva*. Among those, 24 species of bacteria and one species of fungi are known to be pathogenic to either humans, plants, or other animals including *Streptococcus bovis*, *Serratia marcescens*, *Candida* sp., and *Klebsiella variicola*. These species may represent a portion of the natural microbiome associated with *N. fulva*. Furthermore, as microbial species are identified as pathogenic or mutualistic with their host, potential biological control agents may be revealed. Cordycipitaceae, a family of endoparasitic fungi found in insects, was identified in small amounts (0.03%) from the

H2 sampling site. Isolation and identification of this species may yield a potential biological control agent for *N. fulva*.

Tables 12 through 15 summarize the biodiversity of microbiota between sampling sites. Tables 12 and 13 were not standardized and therefore did not take into consideration the amount of template DNA used from each sample. This allowed comparisons between standardized and unstandardized data. Tables 12 and 13 show that ants from H1 had 249 species of bacteria and 52 species of fungi, which is the second highest species count for all sampling sites. Yet, when the amount of template DNA was considered, H1 was the least biodiverse sampling site (Tables 14 and 15). In regards to ectobacterial biodiversity, the rural landscape (L1) was the most rich (51.08 OTUs), followed by R1 (33.46 OTUs), and H2 (28.50 OTUs), and H1 (4.27 OTUs). Pathogenic bacteria species were much more similar between L1 (11.08 OTUs), R1 (11.54 OTUs) and H2 (9.81 OTUs), and highly dissimilar to H1 (1.40 OTUs). Ants collected from hospitals were originally hypothesized to have the highest microbial diversity, at least in regards to pathogenic species. Had samples been recovered from the interior of hospitals, these numbers may have been reversed.

Fungal biodiversity had a very similar trend in regards to overall biodiversity and pathogenic species biodiversity with L1 being the most species rich (13.85 OTUs), followed by H2 (6.48 OTUs), R1 (3.85 OTUs), and H1 (0.91 OTUs). Although the microbiome of *N. fulva* from the rural landscape was more biodiverse, many pathogenic bacterial and fungal species were novel to the hospitals and convalescent home. The juxtaposition of *N. fulva* to medically sensitive environments warrants the consideration

of their medical importance as a result of their propensity to acquire novel pathogens and subsequently deposit them several meters from the point of acquisition. Agroecosystems are also potentially subject to plant disease agent transmission by ants, especially in regards to fungal pathogens. Multiple *Fusarium* and *Gibberella* species were identified from the L1 site. These bacteria cause plant diseases such as Fusarium wilt in many species and dry rot in potatoes; however, only one species of *Fusarium* and *Gibberella* was found on samples from H1 (0.01% of the sample for each species) and R1 (Fusarium = 0.07% of the sample). This suggested that *N. fulva* may be more subjected to a wider diversity of plant pathogens in rural landscapes than in urban environment.

The presence of some bacterial species such as *Lactococcus* (ubiquitous among samples up to 10.37%), *Spiroplasma* (<1% among all sample sights), and *Wolbachia* (0.08% only in the H1 sample site) suggests that some endobacteria may have been unintentionally sampled in this assay. *Lactococcus* occurred among the greatest number of bacterial species for all sample sites (31.91%, 47.82%, 38.92%, and 29.03% for H1, H2, R1, and L1 respectively) just under *Leuconostoc citreum* (31.91% - 47.82% of all samples). The technical difficulty of isolating nucleic acids from the ectomicrobiome was challenging, and although our method likely minimized isolation of nucleic acids from the endomicrobiome, some internal microbes were included in the results. Additionally, some internal microbial species were likely present on the exoskeleton as contaminants from the elimination of wastes, and still other internal microbial species may have the ability to colonize portions of the exoskeleton for some duration.

Nylanderia fulva are difficult to eliminate and an integrated pest management approach is warranted in both medically sensitive areas and agroecosystems. Current management strategies include contact insecticides such as Termidor® (fipronil, sprayed 0.91 m up and 3.05 m out from the exterior of structures), Phantom® (a crack and crevice spray for the interior of structures), and Top Choice® (fipronil granules broadcast throughout lawns and sod farms), as well as bait products such as Advance® Carpenter Ant Bait (abamectin, broadcast around residential and commercial properties and non-occupied patient areas of hospitals and nursing homes; see chapter 3). For hospitals in particular, effective indoor bait stations should be placed at trails where they will be readily discovered. Spinosad is an organic product that can be tested for use in garden crops such as pome fruit, citrus, and leafy and fruiting vegetables. Bait formulations utilizing insect growth regulators are likely to be the most efficacious products for *N. fulva* population management strategies if matrices are attractive to *N. fulva*. Sanitization practices such as the removal of food sources are critical. Cultural practices, for instance, the removal of unnecessary landscape articles that can be used as harborage, and the reduction of unnecessary accumulation of moisture, are also encouraged.

The results of these trials provide evidence that *N. fulva* are potential vectors of *E. coli* and similar pathogens in sensitive environments such as hospitals and convalescent homes. To our knowledge, this is the first of 454 pyrosequencing to determine the microbiota assemblages exclusively on the exterior of arthropods, and provides a model protocol for future studies.

CHAPTER VII

CONCLUSIONS

As the second greatest threat to biodiversity, the mitigation of invasive species is paramount in the preservation of ecosystems. With clarification of the species name of *Nylanderia fulva*, research efforts can now be focused on rapid detection and improved integrated pest management strategies. Continued investigations of *N. fulva* biology and behavior should be pursued in the spirit of applied research. This dissertation investigated an improved method for extracting ants from their substrate, the effectiveness of the sole palatable ant bait on *N. fulva* populations, the seasonal diet preference, peak foraging activity of workers, the fecundity of queens, and the vector potential of this incredible ecological and economically important pest ant species. These projects will expand a base of knowledge upon which many experiments can be built.

The modified drip technique provides an efficient method of extracting ant species from their associated substrates for future laboratory studies. This technique has proven effective for *N. fulva*, *Linepithema humile*, and *Paratrechina longicornis*. Improved rearing techniques, laboratory diets, and transfer procedures that minimize colony mortality would contribute to future experimental methodologies.

Although Advance® Carpenter Ant Bait (ACAB), did not provide adequate population management within our studies, it is evident that ants are attracted to the bait matrix and that subsequent ingestion does result in mortality of workers. Forthcoming

baiting experiments should utilize small infestations as replicates, where the entire population of ants is used as a single treatment. The addition of an insect growth regulator to the ACAB matrix may also be the key to complete population management. Thorough treatments underneath all objects utilized by ants for harborage are also recommended.

Diet preference experiments revealed that *N. fulva* is most attracted to carbohydrate rich diets with high moisture content. This information may lead to improved bait matrix alternatives. Further investigations of actual *N. fulva* food sources within their introduced range will be necessary. These studies may provide data regarding the impact of *N. fulva* on native species, as well as insight into their behavior and relationships with hemipteran insects. Diel foraging behavior was observed when temperatures were between 9.95 - 37.26°C with peak foraging activity occurring at 28.24 ± 3.12°C. Relative abundance data collection should be focused around peak foraging period whenever possible.

A laboratory investigation of *N. fulva* suggested that as the number of queens increased, individual queen fecundity increased. This phenomenon is a novel observation among ants and suggests an alternative mechanism for intracolony dominance. These experiments should be expanded upon to determine if a single queen dominates polygyne colonies or if all queens are increasing egg production. Furthermore, alternative experimental parameters should be explored to determine maximum egg production by queens.

The microbiota assemblages and mechanical vector potential of *N. fulva* establishes this species as a concern for medically sensitive environments. Additional collections of *N. fulva* and other species within hospitals, and subsequent pyrosequencing of their exterior microbiome may reveal definitive patterns of microbiota assemblages of ant species, and reveal their true potential for translocating pathogenic microorganisms. Candidate biological control agents may also be revealed during these experiments. The minimum concentration of microorganisms necessary for their translocation by *N. fulva*, as well as the maximum distance of transfer, should also be determined.

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APPENDIX A

Operational taxonomic units (OTUs)	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Acetobacter orientalis	0.00	0.00	0.00	0.32
Achromobacter subsp. *Xylosoxidans*	0.01	0.00	0.00	0.09
Acidimicrobium ferrooxidans	0.03	0.00	0.04	0.70
Acidithiobacillus caldus	0.01	0.00	0.00	0.00
Acidomonas baliensis	0.04	0.18	0.10	**1.50**
Acidovorax caeni	0.03	0.32	0.00	0.01
Acidovorax delafieldii	0.06	0.04	0.00	0.00
Acidovorax facilis	0.73	0.69	0.23	0.69
Acidovorax temperans	0.80	0.16	0.41	0.07
Acinetobacter calcoaceticus	0.04	0.00	0.01	0.26
Acinetobacter genomosp. 3	0.36	0.09	0.14	0.06
Acinetobacter guillouiae	0.01	0.00	0.00	0.00
Acinetobacter gyllenbergii	0.06	0.19	0.01	0.01
Acinetobacter haemolyticus	0.46	0.07	0.20	0.00
Acinetobacter johnsonii	**2.81**	**1.18**	0.55	0.32
Acinetobacter piperi sp. *novum*	0.05	0.04	0.00	0.04
Acinetobacter rhizosphaerae	0.03	0.02	0.01	0.00
Acinetobacter schindleri	0.00	0.00	0.00	0.16
Actinocorallia sarraceniospora aurea	0.00	0.00	0.02	0.00
Actinokineospora amycolatopsis fastidiosa	0.00	0.00	0.00	0.03
Actinomadura chokoriensis	0.00	0.00	0.00	0.25
Actinomadura vinacea	0.00	0.00	0.00	0.07
Actinomyces canis	0.00	0.04	0.00	0.00
Actinomyces odontolyticus	0.01	0.00	0.00	0.00
Actinomyces sp. oral taxon 848	0.00	0.04	0.00	0.00
Actinomyces urogenitalis	0.04	0.00	0.04	0.03
Actinomyces vaccimaxillae	0.03	0.00	0.00	0.00
Actinoplanes durhamensis	0.00	0.00	0.00	0.06
Actinoplanes liguriae	0.00	0.00	0.00	0.11
Actinoplanes purpeobrunneus	0.00	0.00	0.00	0.03
Actinoplanes rutilosporangius	0.00	0.00	0.00	0.13
Actinotalea fermentans	0.00	0.07	0.15	0.00
Aeromicrobium ginsengisoli	0.01	0.07	0.12	0.06
Aeromicrobium kwangyangensis	0.00	0.00	0.00	0.09
Aeromicrobium marinum	0.00	0.32	0.00	0.04

Aeromicrobium tamlensis	0.00	0.00	0.00	0.03
Aeromonas veronii	0.25	0.19	0.19	0.08
Afipia broomeae	0.00	0.00	0.04	0.20
Agrobacterium larrymoorei	0.03	0.00	0.00	0.00
Agrobacterium tumefaciens	0.59	0.62	0.64	0.11
Agrobacterium vitis	0.02	0.00	0.00	0.00
Agrococcus jenensis	0.00	0.00	0.00	0.28
Agrococcus versicolor	0.04	0.00	0.00	0.00
Agromyces luteolus	0.00	0.00	0.00	0.18
Agromyces ulmi	0.00	0.00	0.00	0.08
Akkermansia muciniphila	0.01	0.00	0.00	0.00
Algoriphagus chordae	0.00	0.00	0.00	0.09
Algoriphagus dokdonensis	0.00	0.00	0.10	0.00
Algoriphagus ratkowskyi	0.00	0.00	0.20	0.00
Algoriphagus winogradskyi	0.00	0.11	0.00	0.00
Alicyclobacillus pohliae	0.00	0.00	0.00	0.09
Aminobacter aminovorans	0.00	0.00	0.00	0.03
Amycolatopsis orientalis	0.00	0.00	0.00	0.05
Amycolatopsis keratiniphila nogabecina	0.00	0.00	0.00	0.34
Amycolatopsis lactamdurans	0.00	0.00	0.00	0.09
Amycolatopsis orientalis	0.00	0.00	0.00	0.62
Amycolatopsis thermoflava	0.00	0.00	0.00	0.06
Anabaena cylindrica	0.00	0.42	0.17	0.00
Anabaenopsis cyanospira rippkae	0.00	0.00	**1.20**	0.00
Anaerococcus octavius	0.01	0.05	0.00	0.15
Anaeromyxobacter dehalogenans	0.00	0.00	0.00	0.02
Anaeromyxobacter sp. fw109_5	0.01	0.00	0.00	0.00
Aphanizomenon issatschenkoi	0.00	0.00	0.22	0.00
Archangium cystobacter violaceus	0.00	0.05	0.00	0.01
Arcobacter butzleri on_107_00	0.37	0.00	0.01	0.06
Arcobacter cryaerophilus	0.03	0.00	0.01	0.00
Arcobacter nitrofigilis	0.03	0.51	0.00	0.00
Arthrobacter chlorophenolicus	0.01	0.02	0.00	0.03
Arthrobacter crystallopoietes	0.00	0.00	0.00	0.06
Arthrobacter globiformis	0.00	0.18	0.00	0.03
Arthrobacter ilicis	0.00	0.02	0.00	0.04
Arthrobacter mysorens	0.00	0.00	0.00	0.65
Arthrobacter nicotianae	0.02	0.00	0.15	0.00
Arthrobacter oxydans	0.00	0.00	0.00	0.12

Arthrobacter ureafaciens	0.01	0.00	0.00	0.26
Azospirillum amazonense	0.00	0.07	0.00	0.00
Bacillus agaradhaerens	0.00	0.00	0.08	0.00
Bacillus amyloliquefaciens	0.00	0.00	0.18	0.01
Bacillus badius	0.00	0.00	0.02	0.00
Bacillus boroniphilus	0.00	0.00	0.00	0.18
Bacillus clausii	0.00	0.00	0.00	0.04
Bacillus firmus	0.04	0.00	0.00	0.00
Bacillus psychrodurans	0.00	0.00	0.02	0.00
Bacillus subtilis sh34	0.01	0.02	0.00	0.00
Bacillus thuringiensis serovar konkukian	0.00	0.00	0.01	0.06
Bacillus vireti	0.00	0.00	0.00	0.02
Bacteroides eggerthii	0.04	0.04	0.00	0.01
Bartonella elizabethae	0.00	0.00	0.22	0.00
Bdellovibrio bacteriovorus	0.00	0.00	0.04	0.00
Beijerinckia derxii venezuelae	0.00	0.00	0.00	0.02
Bifidobacterium minimum	0.03	0.00	0.00	0.10
Bifidobacterium psychraerophilum	0.00	0.00	0.05	0.01
Bilophila wadsworthia	0.00	0.00	0.02	0.01
Blastochloris sulfoviridis	0.00	0.00	0.00	0.12
Blastochloris viridis	0.00	0.00	0.00	0.09
Blastococcus aggregatus	0.00	0.02	0.00	0.13
Bordetella petrii	0.00	0.00	0.00	0.33
Bosea thiooxidans	0.00	0.00	0.00	0.02
Brachybacterium alimentarium	0.06	0.00	0.00	0.00
Brachybacterium paraconglomeratum	0.01	0.00	0.00	0.00
Bradyrhizobium elkanii	0.00	0.00	0.00	0.09
Bradyrhizobium sp. ors278	0.05	0.00	0.00	0.01
Brevibacterium casei	0.00	0.00	0.00	0.20
Brevundimonas alba	0.02	0.00	0.00	0.02
Brevundimonas kwangchunensis	0.00	0.28	0.00	0.02
Brevundimonas vesicularis	0.00	0.04	0.00	0.01
Burkholderia ferrariae	0.02	0.00	0.00	0.01
Burkholderia vietnamiensis	0.00	0.00	0.00	0.04
Candidatus haliscomenobacter calcifugiens	0.05	0.00	0.00	0.00
Candidatus koribacter versatilis	0.00	0.04	0.00	0.00
Candidatus microthrix	0.00	0.09	0.11	0.85
Candidatus solibacter usitatus	0.00	0.00	0.00	0.03
Cardiobacterium hominis	0.00	0.00	0.08	0.00

Catellatospora sp.	0.00	0.00	0.00	0.17
Caulobacter vibrioides	0.07	0.00	0.00	0.12
Cellulosimicrobium cellulans	0.01	0.00	0.05	0.00
Cellulosimicrobium funkei	0.00	0.00	0.17	0.00
Chelativorans multitrophicus	0.00	0.00	0.00	0.45
Chitinophaga arvensicola	0.02	0.37	0.00	**1.26**
Chitinophaga flexibacter	0.11	0.87	0.84	**1.59**
Chloroflexus aurantiacus	0.00	0.00	0.07	0.04
Chondromyces pediculatus	0.00	0.00	0.00	0.02
Chryseobacterium bovis	0.34	0.18	0.12	0.13
Chryseobacterium gregarium	0.02	0.00	0.00	0.00
Chryseobacterium hominis	0.01	0.00	0.01	0.00
Chryseobacterium indologenes	0.08	0.00	0.03	0.00
Chryseobacterium isbiliense	0.10	0.14	0.00	0.00
Chryseobacterium joostei	0.04	0.00	0.02	0.01
Chryseobacterium soldanellicola	0.01	0.00	0.00	0.00
Chthoniobacter flavus	0.00	0.07	0.00	0.09
Citrobacter freundii	**3.57**	**1.63**	**1.26**	0.58
Clostridium baratii	0.00	0.00	0.00	0.11
Clostridium botulinum c	0.03	0.04	0.00	0.00
Clostridium butyricum	0.03	0.00	0.00	0.00
Clostridium chromoreductans	0.00	0.00	0.06	0.00
Clostridium hveragerdense	0.00	0.00	0.00	0.04
Clostridium straminisolvens	0.02	0.00	0.00	0.00
Clostridium subterminale	0.00	0.11	0.00	0.00
Clostridium thermocellum	0.00	0.00	0.00	0.28
Comamonas koreensis	0.01	0.00	0.00	0.02
Comamonas nitrativorans	0.07	0.00	0.00	0.00
Comamonas terrigena	0.09	0.00	0.00	0.01
Comamonas testosteroni sb4	0.03	0.00	0.00	0.01
Congregibacter litoralis	0.00	0.00	0.00	0.07
Corynebacterium tuberculostearicum	0.01	0.04	0.00	0.00
Cupriavidus necator	0.04	0.00	0.00	0.01
Cupriavidus taiwanensis	0.00	0.14	0.00	0.00
Cystobacter ferrugineus	0.00	0.00	0.00	0.21
Cytophaga hutchinsonii	0.00	0.00	0.00	0.09
Dechloromonas spp.	0.06	0.19	0.00	0.00
Dehalococcoides ethenogenes	0.00	0.00	0.00	0.09
Dehalococcoides sp. bav1	0.00	0.19	0.00	0.04

Deinococcus aquaticus	0.03	0.00	0.00	0.00
Deinococcus murrayi	0.01	0.00	0.00	0.00
Deinococcus proteolyticus	0.00	0.05	0.00	0.00
Delftia spp.	0.03	0.58	0.13	0.00
Desulfomicrobium terraneus	0.00	0.07	0.00	0.00
Desulfotalea psychrophila	0.00	0.00	0.00	0.02
Desulfovibrio marrakechensis	0.00	0.00	0.00	0.10
Desulfuromonas thiophila	0.00	0.05	0.00	0.00
Devosia ginsengisoli	0.01	0.00	0.00	0.07
Devosia neptuniae	0.00	0.00	0.00	0.06
Devosia riboflavina	0.00	0.00	0.00	0.67
Devosia soli	0.00	0.00	0.00	0.02
Dialister succinatiphilus	0.07	0.00	0.00	0.00
Dokdonella koreensis	0.08	0.05	0.41	0.01
Ectothiorhodospira shaposhnikovii	0.00	0.00	0.00	0.10
Edwardsiella tarda	0.56	0.00	0.00	0.46
Enhydrobacter aerosaccus	0.31	0.02	0.01	0.04
Ensifer sinorhizobium terangae	0.00	0.00	0.20	0.00
Enterobacter cowanii	0.18	0.00	0.03	0.03
***Enterobacter* sp. 638**	0.28	0.05	0.07	0.09
Enterococcus italicus	0.10	0.11	0.15	0.01
Enterococcus saccharolyticus	0.39	0.07	0.36	0.06
Enterococcus saccharominimus	0.67	0.02	0.84	0.17
Erwinia soli	0.01	0.00	0.00	0.00
Erysipelothrix inopinata	0.03	0.04	0.00	0.00
Erysipelothrix rhusiopathiae	0.04	0.00	0.00	0.00
Erythrobacter piscidermidis	0.00	0.00	0.00	0.13
Eubacterium infirmum	0.01	0.00	0.00	0.00
Flavobacterium columnare	0.05	0.00	0.19	0.00
Flavobacterium denitrificans	0.00	0.00	0.00	0.03
Flavobacterium hydatis	0.04	0.00	0.00	0.00
Flavobacterium johnsoniae uw101	0.01	0.00	0.00	0.11
Flavobacterium succinicans	0.03	0.02	0.00	0.01
Flexibacter flexilis	0.00	0.07	0.00	0.00
Francisella tularensis fsc147 *mediasiatica*	0.00	0.00	0.00	0.04
Frankia sp. cci3	0.00	0.07	0.00	0.00
Frankia sp. ean1pec	0.00	0.32	0.00	0.28
***Geitlerinema* spp.**	0.76	0.02	0.56	0.20
Gemmata obscuriglobus	0.00	0.26	0.00	0.00

Geobacillus thermodenitrificans	0.00	0.34	0.14	0.00
Geobacillus thermoparaffinivorans	0.00	0.00	0.00	0.31
Geodermatophilus blastococcus saxobsidens	0.00	0.00	0.00	0.23
Geodermatophilus obscurus	0.06	0.00	0.00	0.00
Georgenia spp.	0.01	0.00	0.00	0.00
Gloeotrichia echinulata	0.13	0.00	0.00	0.00
Gluconobacter cerinus	0.00	0.00	0.00	0.06
Gluconobacter oxydans sri1995	0.01	0.00	0.00	0.01
Gluconobacter spp.	0.02	0.00	0.01	**1.44**
Glycomyces harbinensis	0.00	0.00	0.00	0.03
gn02 (candidate division)	0.01	0.00	0.00	0.00
Gordonia rubripertincta	0.00	0.00	0.01	0.01
Granulicatella elegans	0.01	0.00	0.00	0.00
Granulicatella paradiacens	0.00	0.00	0.34	0.00
Haliangium spp.	0.03	0.35	0.00	0.04
Herbaspirillum frisingense	0.07	0.00	0.00	0.00
Herbaspirillum putei	0.00	0.02	0.17	0.06
Herbaspirillum seropedicae	0.00	0.00	0.00	0.03
Hydrocarboniphaga spp.	0.00	0.00	0.00	0.10
Hydrogenophaga palleronii	0.00	0.00	0.29	0.00
Hymenobacter spp.	0.00	0.00	0.00	0.02
Hyphomicrobium zavarzinii	0.02	0.00	0.00	0.23
Isoptericola variabilis	0.00	0.00	0.23	0.00
Janibacter spp.	0.00	0.00	0.00	0.11
Janthinobacterium lividum	0.05	0.00	0.00	0.00
Janthinobacterium spp.	0.03	0.00	0.02	0.01
Kaistobacter spp.	0.02	0.18	0.00	0.56
Kineococcus radiotolerans	0.00	0.00	0.00	0.28
Klebsiella oxytoca	**3.60**	**1.11**	**1.24**	0.72
Klebsiella pneumoniae 521	0.01	0.00	0.01	0.00
Klebsiella variicola	0.40	0.16	0.35	0.21
Knoellia spp.	0.00	0.00	0.00	0.03
Knoellia subterranea	0.00	0.00	0.00	0.21
Kocuria marina	0.00	0.02	0.00	0.27
Kocuria rosea	0.00	0.00	0.02	0.00
Kribbella karoonensis	0.00	0.00	0.00	0.12
Kribbella sandramycini	0.00	0.00	0.00	0.10
ksb1 (candidate division)	0.00	0.09	0.00	0.00
Lactobacillus acidophilus	0.00	0.00	0.00	0.24

Lactobacillus delbrueckii	0.20	0.07	0.00	0.00
Lactobacillus fermentum	0.46	0.42	0.65	0.23
Lactobacillus intestinalis	0.03	0.00	0.00	0.00
Lactobacillus paracasei	0.01	0.00	0.00	0.00
Lactobacillus plantarum	0.02	0.00	0.00	0.00
Lactobacillus sanfranciscensis	0.31	0.21	0.00	0.02
Lactococcus lactis	**7.17**	**3.94**	**5.30**	**2.84**
Lactococcus piscium	0.02	0.00	0.00	0.00
Lactococcus plantarum	**10.37**	**6.69**	**7.00**	**3.84**
Lactococcus raffinolactis	**4.72**	**3.39**	**3.53**	**2.03**
Leclercia adecarboxylata	0.59	0.16	0.23	0.12
Lentzea albida	0.00	0.00	0.00	0.33
Lentzea waywayandensis	0.00	0.00	0.00	0.13
Leptolyngbya frigida	0.00	0.25	**1.37**	0.00
Leptolyngbya rozosum	0.00	0.00	0.30	0.00
Leptothrix discophora	0.00	0.00	0.00	0.09
Leptotrichia trevisanii	0.01	0.00	0.00	0.00
Leuconostoc citreum	**31.91**	**47.82**	**38.97**	**29.03**
Leuconostoc fallax	0.45	0.53	0.07	0.18
Leuconostoc kimchii	0.01	0.00	0.01	0.00
Leuconostoc lactis	0.57	0.76	0.70	0.52
Lysobacter enzymogenes	0.09	0.02	0.00	0.21
Lysobacter gummosus	0.01	0.00	0.00	0.00
Magnetospirillum sp.	0.02	0.00	0.00	0.00
Mannheimia varigena	0.01	0.00	0.00	0.00
Marinobacter mobilis	0.00	0.00	0.13	0.04
Marinobacter sp. elb17	0.00	0.00	0.03	0.00
Marmoricola spp.	0.00	0.00	0.04	0.65
Massilia timonae	0.00	**1.02**	0.00	0.38
Megasphaera elsdenii	0.00	0.00	0.02	0.00
Meiothermus taiwanensis	0.00	0.00	0.00	0.39
Mesoplasma florum	0.01	0.34	0.04	0.02
Mesorhizobium loti	0.07	0.00	0.09	0.02
Methylibium petroleiphilum	0.06	0.00	0.07	0.01
Methylobacterium adhaesivum	0.00	0.00	0.00	0.04
Methylobacterium chloromethanicum	0.00	0.00	0.00	0.26
Methylobacterium hispanicum	0.01	0.00	0.00	0.00
Methylobacterium isbiliense	0.00	0.00	0.00	0.04
Methylobacterium jeotgali	0.01	0.19	0.00	0.01

Methylobacterium komagatae	0.00	0.00	0.00	0.25
Methylobacterium organophilum	0.15	0.00	0.15	0.11
Methylobacterium radiotolerans	0.01	0.00	0.07	0.00
Methylobacterium sp. 4_46	0.00	0.00	0.26	0.00
Methylobacterium suomiense	0.00	0.00	0.00	0.49
Methylobacterium thiocyanatum	0.00	0.00	0.00	0.09
Methylobacterium zatmanii	0.00	0.05	0.00	0.00
Methylocaldum tepidum	0.00	0.00	0.63	0.00
Methylosinus sporium	0.02	0.00	0.00	0.00
Methylosinus trichosporium	0.00	0.00	0.03	0.04
Methylotenera spp.	0.00	0.05	0.00	0.00
Microbacterium aurum	0.04	0.00	0.00	0.51
Microbacterium flavescens	0.03	0.00	0.00	0.02
Microbacterium hydrocarbonoxydans suf1	0.00	0.00	0.00	0.02
Microbacterium marinilacus	0.00	0.00	0.00	0.02
Microbacterium phyllosphaerae wp02_2_229	0.00	0.00	0.00	0.10
Microbacterium testaceum	0.00	0.00	0.00	0.09
Micrococcus luteus	0.00	0.00	0.00	0.55
Microcoleus chthonoplastes	0.00	0.00	0.02	0.00
Microcystis aeruginosa	0.00	0.00	0.06	0.00
Microcystis ichthyoblabe	0.00	0.00	0.08	0.00
Micromonospora carbonacea	0.00	0.00	0.00	0.02
Micromonospora fulviviridis	0.00	0.00	0.00	0.19
Micromonospora lupini	0.00	0.00	0.42	0.05
Micromonospora matsumotoense	0.00	0.00	0.00	0.04
Micromonospora mirobrigensis	0.00	0.05	0.00	0.00
Microvirgula aerodenitrificans	**2.04**	0.53	0.69	0.47
Morganella morganii	0.07	0.00	0.03	0.00
Mycobacterium austroafricanum vm0573	0.00	0.21	0.00	0.00
Mycobacterium avium complex	0.00	0.00	0.00	0.04
Mycobacterium holsaticum	0.00	0.12	0.00	0.00
Mycobacterium immunogenum	0.00	0.00	0.00	0.05
Mycobacterium lacticola	0.01	0.00	0.00	0.00
Mycobacterium neglectum	0.00	0.00	0.00	0.11
Myxococcus xanthus	0.00	0.00	0.00	0.05
Natronobacillus azotifigens	0.00	0.00	0.00	0.09
nc10 (candidate division)	0.09	0.99	0.04	0.46
Nesterenkonia halophila	0.00	0.00	0.00	0.13

Nitrosospira multiformis	0.00	0.00	0.00	0.03
Nitrospira moscoviensis	0.00	0.09	0.00	0.28
Nocardia concava	0.00	0.00	0.00	0.03
Nocardia cyriacigeorgica	0.00	0.00	0.00	0.31
Nocardioides albus	0.00	0.00	0.01	0.56
Nocardioides furvisabuli	0.00	0.05	0.00	0.00
Nocardioides kribbensis	0.00	0.00	0.00	0.54
Nocardioides marinisabuli	0.00	0.00	0.04	0.04
Nocardioides oleivorans	0.00	0.07	0.00	**2.16**
Nocardioides plantarum	0.00	0.00	0.01	0.35
Nocardioides sp. js614	0.00	0.00	0.20	0.42
Nocardiopsis kunsanensis	0.02	0.00	0.00	0.18
Novosphingobium pentaromativorans	0.00	0.00	0.00	0.07
***Novosphingobium* spp.**	0.07	0.11	0.07	0.01
Novosphingobium subterraneum	0.03	0.00	0.00	0.01
Ochrobactrum grignonense	0.00	0.07	0.00	0.01
Ochrobactrum pseudogrignonense	0.12	0.02	0.00	0.00
Ochrobactrum tritici tj3	0.01	0.00	0.00	0.13
op11 (candidate division)	0.00	0.00	0.09	0.00
op8 (candidate division)	0.01	0.00	0.12	0.00
Opitutus terrae	0.00	0.39	0.00	0.00
Oscillatoria spp.	0.00	0.00	**3.36**	0.00
Oscillochloris trichoides	0.00	0.00	0.04	0.00
Oscillospira spp.	0.01	0.00	0.00	0.17
Paenibacillus panaciterrae	0.00	0.00	0.15	0.00
Pantoea ananatis	0.01	0.07	0.00	0.00
Paracoccus alcaliphilus	0.01	0.04	0.00	0.26
Paracoccus koreensis	0.02	0.00	0.00	0.00
Paracoccus marcusii	0.21	0.04	0.10	0.00
Paracoccus versutus	0.07	0.00	0.00	0.00
Paracoccus zeaxanthinifaciens	0.00	0.00	0.08	0.01
Pectobacterium wasabiae	0.09	0.00	0.00	0.01
Pedobacter terrae	0.00	0.05	0.00	0.00
Pedomicrobium spp.	0.01	0.00	0.00	0.00
Pelobacter carbinolicus	0.00	0.00	0.00	0.24
Peptoniphilus asaccharolyticus	0.00	0.16	0.00	0.00
Phaeospirillum fulvum	0.00	0.00	0.00	0.03
Phascolarctobacterium spp.	0.00	0.09	0.00	0.00
Phenylobacterium immobile	0.00	0.00	0.00	0.94

Phormidium animale	0.00	0.00	0.84	0.00
Phormidium pristleyi	0.00	0.00	0.78	0.11
Pimelobacter simplex	0.00	0.00	0.39	0.35
Pimelobacter spp.	0.00	0.00	0.00	0.59
Pirellula spp.	0.00	0.00	0.00	0.18
Planctomyces spp.	0.02	0.00	0.00	0.13
Planktothricoides raciborskii	0.00	0.00	0.05	0.00
Planktothricoides spp.	0.00	0.00	**1.08**	0.00
Planktothrix mougeotii	0.00	0.00	0.06	0.00
Pleomorphomonas oryzae	0.01	0.00	0.00	0.00
Plesiocystis spp.	0.07	0.00	0.00	0.00
***Polaromonas* spp.**	0.02	0.11	0.06	0.03
Pontibacter korlensis	0.00	0.00	0.14	0.16
Porphyrobacter spp.	0.00	0.00	0.00	0.05
Porphyrobacter tepidarius	0.00	0.05	0.00	0.48
Prevotella copri	0.16	0.07	0.05	0.01
Prevotella melaninogenica	0.03	0.00	0.01	0.00
Propionibacterium acnes	0.06	**1.54**	**3.19**	0.68
Propionibacterium granulosum	0.01	0.00	0.00	0.05
Propionibacterium microaerophilum	0.02	0.00	0.00	0.00
Propionibacterium propionicum	0.00	0.05	0.00	0.00
Prosthecobacter debontii	0.00	0.00	0.00	0.50
Pseudomonas aeruginosa pao1	0.14	0.00	0.00	0.11
Pseudomonas fulva	0.11	0.07	0.00	0.01
Pseudomonas oryzihabitans	0.01	0.02	0.01	0.00
Pseudomonas putida	0.20	0.04	0.01	0.21
Pseudomonas stutzeri	0.00	0.00	0.08	0.00
Pseudomonas taiwanensis	0.01	0.00	0.00	0.00
Pseudomonas trivialis	0.01	0.00	0.00	0.00
Pseudonocardia endophyticus	0.00	0.04	0.14	0.00
Pseudonocardia halophobica	0.00	0.00	0.00	0.09
Pseudonocardia sulfidoxydans	0.01	0.00	0.00	0.08
Pseudonocardia yunnanensis	0.00	0.19	0.00	0.00
Pseudonocardia zijingensis	0.00	0.00	0.13	0.45
Pseudoxanthomonas mexicana	0.06	0.00	0.00	0.00
Ralstonia pickettii	0.00	0.00	0.01	0.06
Ramlibacter spp.	0.01	0.07	0.13	0.06
Raoultella terrigena	0.30	0.12	0.03	0.05
Rheinheimera chironomi	0.01	0.00	0.01	0.00

Rhizobium huautlense	0.02	0.00	0.01	0.01
Rhizobium taeanense	0.03	0.14	0.00	0.02
Rhizobium yanglingense	0.00	0.07	0.00	0.01
Rhodobacter blasticus	0.00	0.00	0.03	0.00
Rhodobacter sphaeroides	0.07	0.00	0.01	0.00
Rhodobium orientis	0.01	0.00	0.00	0.00
Rhodococcus equi	0.00	0.00	0.02	0.06
Rhodococcus globerulus	0.00	0.00	0.00	0.02
Rhodocyclus tenuis	0.02	0.00	0.00	0.00
Rhodoplanes spp.	0.01	0.16	0.00	0.07
Rickettsia amblyommii	0.03	0.00	0.00	0.00
Rickettsiella grylli	0.00	0.00	0.00	**3.00**
Rickettsiella popilliae	0.18	0.00	0.00	0.00
Riemerella columbina	0.05	0.00	0.00	0.00
Roseomonas cervicalis	0.00	0.00	0.00	0.11
Roseomonas gilardii	0.00	0.35	0.00	0.16
Roseomonas ruber	0.00	0.00	0.06	0.00
Rubellimicrobium spp.	0.00	0.32	0.07	0.18
Rubrobacter radiotolerans	0.01	0.42	0.28	0.04
Rubrobacter xylanophilus	0.01	0.00	0.00	0.04
Saccharomonospora azurea	0.00	0.00	0.00	0.06
Saccharopolyspora gloriosa	0.00	0.00	0.00	0.02
Saccharothrix texasensis	0.00	0.00	0.00	0.09
Sebaldella termitidis	0.00	0.00	0.12	0.06
Segetibacter spp.	0.09	0.07	0.00	0.09
Sejongia spp.	0.02	0.00	0.01	0.01
Serratia marcescens	**1.62**	0.65	0.76	0.83
Serratia plymuthica	0.03	0.00	0.00	0.00
Shinella zoogloeoides	0.00	0.11	0.01	0.00
Singulisphaera acidiphila	0.00	0.00	0.18	0.00
Singulisphaera spp.	0.00	0.00	0.20	0.02
Solibacillus silvestris	0.00	0.00	0.00	0.04
Solimonas soli	0.02	0.00	0.19	0.02
Sorangium cellulosum	0.01	0.00	0.16	0.00
spam (candidate division)	0.00	0.41	0.42	0.44
Sphingobacterium composta	0.04	0.00	0.00	0.00
Sphingobacterium multivorum	0.00	0.00	0.00	0.11
Sphingobacterium thalpophilum	0.00	0.00	0.00	0.04
Sphingobium chlorophenolicum pathovar	0.07	0.00	0.00	0.00

Sphingobium yanoikuyae	0.03	0.37	0.00	0.25
Sphingomonas adhaesiva	0.08	0.00	0.00	0.00
Sphingomonas asaccharolytica	0.05	0.00	0.00	0.22
Sphingomonas faeni	0.00	0.00	0.00	0.11
Sphingomonas melonis	0.05	0.00	0.00	0.00
Sphingomonas phyllosphaerae	0.00	0.00	0.00	0.33
Sphingomonas wittichii	0.01	0.02	0.00	0.43
Sphingomonas yunnanensis	0.09	0.00	0.00	0.00
Sphingopyxis ginsengisoli	0.00	0.00	0.00	0.18
Spiroplasma diminutum	0.01	0.00	0.00	0.00
Spiroplasma lampyridicola	0.04	0.04	0.00	0.33
***Spiroplasma* spp.**	0.49	0.99	0.11	0.19
Spirosoma spp.	0.00	0.48	0.00	0.00
Sporosarcina bacillus ginsengi	0.00	0.00	0.00	0.08
Staphylococcus epidermidis mother c4	0.00	0.32	0.07	0.04
Staphylococcus pasteuri	0.00	0.05	0.01	0.05
Staphylococcus sciuri	0.01	0.00	0.00	0.00
Stenotrophomonas maltophilia	0.09	0.07	0.15	0.18
Streptococcus agalactiae	0.02	0.00	0.00	0.00
Streptococcus bovis	**5.15**	**2.28**	**2.47**	**1.66**
Streptococcus equinus	0.23	0.11	0.11	0.05
Streptococcus lutetiensis	0.01	0.00	0.01	0.00
Streptococcus macedonicus	0.01	0.00	0.00	0.00
Streptococcus parauberis	0.00	0.00	0.00	0.02
Streptomyces aculeolatus	0.00	0.00	0.00	0.02
Streptomyces agglomeratus	0.00	0.00	0.00	0.07
Streptomyces albiaxialis	0.00	0.00	0.00	0.13
Streptomyces brunneogriseus	0.00	0.00	0.00	0.05
Streptomyces cacaoi	0.00	0.00	0.00	0.19
Streptomyces carpinensis	0.00	0.00	0.00	0.02
Streptomyces flavoviridis	0.03	0.00	0.00	0.03
Streptomyces glaucosporus	0.00	0.00	**1.74**	0.00
Streptomyces glomeratus	0.01	0.00	0.00	0.00
Streptomyces heteromorphus	0.00	0.00	0.00	0.06
Streptomyces hiroshimensis	0.00	0.00	0.00	0.03
Streptomyces kunmingensis	0.00	0.00	0.00	0.48
Streptomyces lanatus	0.00	0.00	0.00	0.59
Streptomyces lincolnensis	0.00	0.00	0.00	0.10
Streptomyces malachitospinus	0.00	0.00	0.00	0.02

Streptomyces mirabilis	0.00	0.00	0.00	0.34
Streptomyces nanshensis	0.00	0.00	0.00	0.83
Streptomyces narbonensis	0.00	0.00	0.00	0.03
Streptomyces netropsis	0.00	0.00	0.00	0.07
Streptomyces platensis malvinus	0.00	0.00	0.00	0.09
Streptomyces radiopugnans	0.00	0.00	0.04	0.00
Streptomyces sanglieri	0.00	0.00	0.00	0.10
Streptomyces sclerotialus	0.00	0.00	0.42	0.00
Streptomyces shandonggensis	0.00	0.00	0.00	0.03
Streptomyces tanashiensis	0.00	0.00	0.00	0.13
Streptomyces tenebrarius	0.00	0.00	0.00	0.40
Streptomyces thermoalcalitolerans	0.00	0.00	0.17	0.00
Streptomyces thermovulgaris	0.00	0.00	0.00	0.06
Streptomyces toxytricini	0.05	0.00	0.00	0.00
Streptomyces tritolerans	0.07	0.02	0.00	0.21
Streptomyces violarus	0.00	0.00	0.00	0.06
Streptomyces viridiviolaceus	0.00	0.00	0.00	0.02
Streptomyces viridocyaneus	0.00	0.00	0.00	0.05
Streptomyces yatensis	0.01	0.00	0.00	0.00
Streptosporangium amethystogenes	0.00	0.00	0.12	0.00
Sulfurospirillum spp.	0.14	0.12	0.08	0.01
Sulfurovum spp.	0.01	0.00	0.00	0.00
Terriglobus roseus	0.01	0.00	0.00	0.00
Thermobaculum terrenum	0.00	0.00	0.00	0.04
Thermobispora bispora	0.00	0.05	0.00	0.10
Thermomonas brevis	0.01	0.00	0.00	0.01
Thermomonas dokdonensis	0.05	0.00	0.00	0.08
Thermomonospora chromogena	0.00	0.09	0.00	0.00
Thermosinus carboxydivorans	0.00	0.00	0.11	0.00
Thioalkalivibrio denitrificans	0.00	0.07	0.00	0.01
Thioalkalivibrio thiocyanodenitrificans	0.00	0.00	0.00	0.06
Thiorhodovibrio winogradskyi	0.00	0.42	0.00	0.02
tm6 (candidate division)	0.04	0.00	0.00	0.00
tm7 (candidate division)	0.28	0.35	0.00	0.27
Tolumonas auensis	0.02	0.00	0.02	0.01
Trabulsiella citrobacter farmeri	0.16	0.02	0.06	0.06
Trabulsiella kluyvera ascorbata	0.88	0.19	0.31	0.09
***Trabulsiella* spp.**	0.32	0.07	0.14	0.06
Tsukamurella standjordii	0.00	0.00	0.00	0.99

Vagococcus fluvialis	0.06	0.00	0.00	0.00
Vagococcus salmoninarum	0.02	0.00	0.00	0.00
Variovorax paradoxus	0.13	0.25	0.00	0.04
Veillonella dispar	0.11	0.19	0.02	0.03
Veillonella parvula	**1.97**	**2.19**	0.85	0.70
Victivallis vadensis	0.00	0.00	0.00	0.22
Vogesella indigofera	0.14	0.00	0.20	0.00
Vogesella spp.	0.02	0.00	0.04	0.00
Weissella confusa	**4.51**	**2.76**	**3.55**	**1.76**
Williamsia muralis	0.00	0.00	0.00	0.03
Wolbachia sp.	0.08	0.00	0.00	0.00
ws3 (candidate division)	0.05	0.00	0.00	0.00
Xanthomonas hyacinthi	0.05	0.00	0.00	0.00
Xanthomonas vesicatoria	0.20	0.00	0.00	0.00
Yonghaparkia alkaliphila	0.00	0.00	0.00	0.02
Yonghaparkia palkaliphila	0.00	0.00	0.00	0.04
Zoogloea ramigera	0.00	0.00	0.04	0.00
Total	100.0	100.0	100.0	100.0

The BLASTn hit for each sequence was analyzed separately for each sample providing relative abundance information within and among the individual samples based upon relative numbers of sequences within each. Percentages >1% are indicated in bold for visualization of important bacterial genera or species. Bacterial genera and species in bold identify those that are present in all sample sites.

APPENDIX B

Operational taxonomic units (OTUs)	Hospital (H1)	Hospital (H2)	Retirement Community (R1)	Rural Landscape (L1)
Acremonium sp.	0.00	0.00	0.00	0.04
Acremonium sp. r028	0.00	0.00	0.00	0.64
Agaricales sp.	0.01	0.00	0.00	0.00
Agaricomycetes sp.	0.06	0.00	0.00	0.03
Alternaria alternate	0.10	0.00	0.00	**2.22**
Alternaria sp.	0.02	0.00	0.00	0.36
Alternaria sp. ss_l8	0.02	0.00	0.00	0.19
Ascomycete sp.	0.02	0.00	0.00	0.00
Ascomycete sp. hkc14	0.00	0.00	0.00	0.88
Ascomycota sp.	0.03	0.20	0.00	0.02
Ascomycota sp. morphotype no. 10	0.01	0.00	0.00	0.00
Ascomycota sp. unspecified class unspecified order	0.03	0.00	0.00	**1.30**
Aspergillus aculeatus	0.00	0.00	0.00	0.52
Aspergillus nomius	0.00	0.00	0.00	0.77
Aspergillus sp.	0.00	0.00	0.00	0.43
Aureobasidium sp.	0.02	0.00	0.00	0.00
Auxarthron filamentosum	0.00	0.00	0.00	0.07
Basidiomycota sp.	0.01	0.00	0.00	0.47
***Candida* sp.**	**7.70**	**21.54**	**18.17**	**10.40**
Candida sp. n17	0.01	0.00	0.00	0.00
Candida tropicalis	**21.27**	**15.05**	**47.98**	**6.88**
Capnodiales sp.	0.00	0.00	0.00	0.05
Ceratobasidium sp. ag_a	0.00	0.00	0.10	0.00
Cerrena sp.	0.00	0.00	0.00	0.13
Cerrena sp. pdd 95790	0.00	0.00	0.00	**1.17**
Chaetomium sp.	0.01	0.00	0.00	0.00
Chaetomium sp. *aurim*1195	0.39	0.00	0.00	0.00
Cladosporium cladosporioides	0.00	0.00	0.07	0.53
Cladosporium exasperatum	0.00	0.00	0.00	0.03
Cladosporium sp.	0.00	0.00	0.00	0.04
Cladosporium sphaerospermum	0.00	0.00	0.00	0.64
Cochliobolus lunatus	0.00	**3.71**	0.00	0.09
Coprinellus radians	0.00	0.00	**1.37**	0.52
Cordycipitaceae sp.	0.00	0.03	0.00	0.00
Davidiellaceae sp.	0.00	0.00	0.00	0.06

Didymellaceae sp.	0.00	0.01	0.00	0.00
***Dipodascaceae* sp.**	**26.22**	**19.35**	**4.71**	**19.54**
Dipodascus australiensis	0.75	**2.26**	0.00	0.01
Dipodascus sp.	**3.29**	0.24	0.00	0.00
Dipodascus sp. czc0208	0.07	0.00	0.00	0.00
Dokmaia monthadangii	0.00	0.00	0.00	0.75
Dokmaia sp.	0.00	0.00	0.00	0.05
Dothideomycetes sp.	0.00	**4.86**	0.00	**1.08**
Eupenicillium cinnamopurpureum	0.00	0.00	0.00	0.25
Eurotiales sp.	0.00	0.03	0.00	0.00
Exobasidiomycetidae sp. ibl 03150	0.00	0.00	0.00	0.12
Fusarium equiseti	0.00	0.00	0.00	**1.31**
Fusarium oxysporum	0.00	0.00	0.00	**4.34**
Fusarium solani	0.01	0.00	0.00	**2.24**
Fusarium sp.	0.00	0.00	0.00	0.02
Fusarium sp. 410	0.00	0.00	0.07	0.00
Fusarium sp. jy2	0.00	0.00	0.00	**4.58**
Fusarium sp. p37e1	0.00	0.00	0.00	0.16
Galactomyces geotrichum	**2.72**	0.00	**9.64**	0.02
Galactomyces sp.	**1.42**	0.00	**1.35**	0.01
Galactomyces sp. bpy_54	0.47	0.00	0.00	0.00
Geotrichum gigas	0.49	0.28	0.02	0.23
***Geotrichum* sp.**	**27.69**	**5.35**	**4.16**	**5.99**
Gibberella fujikuroi	0.00	0.00	0.00	**1.57**
Gibberella pulicaris	0.01	0.00	0.00	0.00
Gibberella sp.	0.00	0.00	0.00	0.30
Gibberella sp. bf22	0.00	0.00	0.00	0.01
Humicola sp. l_2	0.00	0.00	**2.43**	0.00
Hypocreales sp.	0.00	0.02	0.00	0.41
Hypocreales sp. 2 mj17	0.00	0.00	0.00	0.58
Hypocreales sp. ibl 03161	0.00	0.00	0.00	0.78
Hypocreales sp. lm153	0.00	0.00	0.00	0.02
Hypocreales sp. lm85	0.00	0.00	0.00	**1.99**
Hypocreales sp. unspecified family	0.00	**1.02**	0.00	0.75
Leotiomycetes sp. unspecified order	0.00	0.03	0.00	0.00
Malassezia restricta	0.00	0.02	0.00	0.07
Malassezia sp.	0.01	0.00	0.00	0.00
Microascaceae sp.	0.01	0.00	0.00	0.00
Mortierella alpina	0.00	0.00	0.00	0.63

Mortierella sp. tufc 20030	0.00	0.02	0.00	0.00
Mycosphaerella sp.	0.01	0.00	0.00	0.00
Mycosphaerella sp. mec1	0.08	0.00	0.00	0.00
Mycosphaerellaceae sp.	0.00	0.00	0.00	0.08
Mycosphaerellaceae sp. lm483	0.00	0.00	0.00	0.30
Myrmecridium sp. asr_306	0.01	0.00	0.00	0.00
Myrothecium atroviride	0.00	0.42	0.00	0.00
Myrothecium atrum	0.00	**3.82**	0.00	0.00
Myrothecium gramineum	0.00	**8.55**	0.00	0.00
Myrothecium roridum	0.00	0.03	0.00	**1.02**
Myrothecium sp.	0.00	0.79	0.00	0.00
Myrothecium sp. 2 tms_2011	0.00	**4.81**	0.02	0.01
Myrothecium verrucaria	0.00	**3.86**	0.00	0.01
Nectria inventa	0.02	0.00	0.00	0.00
Nectriaceae sp.	0.00	0.00	0.00	0.17
Paecilomyces marquandii	0.00	0.00	0.00	0.15
Paraphoma fimeti	0.00	0.00	0.00	0.08
Penicillium citrinum	0.00	0.00	0.00	0.95
Penicillium sp.	0.00	0.00	0.00	0.25
Penicillium sp. lh185	0.00	0.00	0.00	0.05
Pezizales sp.	0.00	0.00	0.00	0.70
Phoma herbarum	0.00	0.01	0.00	0.00
Phoma sp.	0.00	0.38	0.00	0.00
Phoma sp. p8e5	0.00	**2.22**	0.00	0.00
Physisporinus vitreus	0.00	0.00	0.00	0.12
Plectosphaerella sp.	0.00	0.00	0.43	0.54
Plectosphaerella sp. fpglxj03	0.00	0.00	**4.95**	0.03
Plectosphaerellaceae sp.	0.00	0.00	0.02	0.01
Pleosporaceae sp.	0.00	0.24	0.00	0.07
Pleosporales sp.	0.00	0.00	0.00	0.74
Plicaria anthracina	0.00	0.00	0.00	**4.02**
Podospora decipiens	0.00	0.09	0.00	0.00
Pseudozyma aphidis	0.00	0.00	0.00	0.03
Ramalinaceae sp.	0.00	0.01	0.00	0.00
Rhizophydiales sp.	0.21	0.00	0.00	0.00
Rhodotorula glutinis	0.22	0.00	0.00	0.00
Robillarda sessilis	0.00	0.00	0.00	0.61
***Saccharomycetales* sp.**	**2.18**	0.71	**3.46**	**4.13**
Saccharomycetes sp.	0.02	0.01	0.00	0.01

Sarocladium sp.	0.00	0.00	0.00	**3.74**
Scedosporium apiospermum	0.00	0.00	0.00	0.00
Thanatephorus cucumeris	0.04	0.00	0.00	0.00
Trametes cubensis	0.00	0.00	0.00	**1.51**
Trametes lactinea	0.00	0.03	0.00	0.01
Trametes sp.	0.00	0.00	0.00	0.94
Tremellales sp. unspecified family	0.14	0.00	0.00	0.00
Trichocladium asperum	0.02	0.00	0.00	0.00
Trichocladium sp.	0.13	0.00	0.00	0.00
Trichocomaceae	0.00	0.00	0.00	0.25
Trichocomaceae sp. lm114	0.00	0.00	0.00	0.40
Trichosporon asahii	0.04	0.00	0.00	0.00
Trichosporon domesticum	0.01	0.00	0.00	0.00
Trichosporon guehoae	0.20	0.00	0.91	0.00
Trichosporon moniliiforme	**2.78**	0.00	0.00	0.00
Trichosporon mucoides	0.34	0.00	0.00	0.00
Trichosporon sp.	0.66	0.00	0.00	0.00
Trichosporonales sp. lm117	0.02	0.00	0.00	0.00
Verticillium sp.	0.00	0.00	0.12	0.00
Volutella sp.	0.00	0.00	0.00	**1.02**
Wallemia sp. f53	0.00	0.00	0.00	0.16
Wallemiomycetes sp.	0.00	0.00	0.00	**1.62**
Total	100.0	100.0	100.0	100.0

The BLASTn hit for each sequence was analyzed separately for each sample providing relative abundance information within and among the individual samples based upon relative numbers of sequences within each. Percentages >1% are indicated in bold for visualization of important fungal genera or species. Fungal genera and species in bold identify those that are present in all sample sites.